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(54) Title: TETRODOTOXIN-SENSITIVE SODIUM CHANNEL α -SUBUNIT (57) Abstract DNA encoding for a voltage-gated, TTX-sensitive sodium channel is isolated. Also disclosed are polypeptide products of recombinant expression of these DNA sequences, expression vectors comprising the DNA sequence, and host cells transformed with these expression vectors. Other aspects of this invention are peptides whose sequences are based on the amino acid sequences deduced from these DNA sequences, antibodies specific for such proteins and peptides, procedures for detection and quantitation of such proteins and nucleic acids related thereto. Another aspect of this invention is the use of this voltage-gated, tetrodotoxin-sensitive sodium channel as a therapeutic target for compounds.		

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- 1 -

Tetrodotoxin-Sensitive Sodium Channel α -Subunit

The present invention relates generally to sodium channel proteins and more particularly to a novel cloned α -subunit of a voltage-gated, tetrodotoxin-sensitive sodium channel protein. The present invention further relates to its production by recombinant technology and nucleic acid sequences encoding for this protein.

BACKGROUND OF THE INVENTION

The basic unit of information transmitted from one part of the nervous system to another is a single action potential or nerve impulse. The „transmission line“ for these impulses is the axon, or nerve fiber. The electrical excitability of the nerve membrane has been shown to depend on the membrane's voltage-sensitive ionic permeability system that allows it to use energy stored in ionic concentration gradients. Electrical activity of the nerve is triggered by a depolarization of the membrane, which opens channels through the membrane that are highly selective for sodium ions, which are then driven inward by the electrochemical gradient. Of the many ionic channels, the voltage-gated or voltage-sensitive sodium channel is one of the most studied. It is a transmembrane protein that is essential for the generation of action potentials in excitable cells. An excellent review of sodium channels is presented in Catterall, TINS 16(12), 500-506 (1993).

The cDNAs for several Na^+ channels have been cloned and sequenced. Numa *et al.*, Annals of the New York Academy of Sciences 479, 338-355 (1986), describe cDNA from the electric organ of eel and two different ones from rat brain. Rogart, U.S. Patent No. 5,380,836, describes cDNA from rat cardiac tissue. See also Cribbs *et al.* Proc. Natl. Acad. Sci., 86, 8170-8174 (1989). A peripheral nerve sodium channel, referred to as PN1, has been detected based on sodium current studies and hybridization to a highly conserved sodium channel probe by D'Arcangelo *et al.*, J. Cell Biol. 122, 915-921 (1993). However, neither the DNA nor the protein were isolated and its complete nucleic acid and amino acid sequence were unidentified. A partial amino acid sequence was presented at the 23rd Annual Meeting of the Society for Neuroscience, November 7-12, 1993, Washington D.C., see Abstracts: Volume 19, Part 1: Abstract 121.7: "Nerve Growth Factor Treatment of PC12 Cells Induces the Expression of a Novel Sodium Channel Gene, Peripheral Nerve Type 1 (PN1)", by B.L. Moss, J. Toledo-Aral and G. Mandel.

A sodium channel gene abundantly expressed in neurons and glia, referred to as NaCh6, was detected in 1995 based on RNase protection assays, *in situ* hybridization and RT-PCR hybridization. See Schaller *et al.*, J. Neuroscience 15(5), 3231-3242 (1995).

5 These studies have shown that the amino acid sequence of the Na⁺ channel has been conserved over a long evolutionary period. These studies have also revealed that the channel is a single polypeptide containing four internal repeats, or homologous domains (domains I-IV), having similar amino acid sequences. Each domain folds into six predicted
10 transmembrane α -helices or segments: five are hydrophobic segments and one is highly charged with many lysine and arginine residues. This highly charged segment is the fourth transmembrane segment in each domain (the S4 segment) and is likely to be involved in voltage-gating. The positively charged side chains on the S4 segment are likely to be paired
15 with the negatively charged side chains on the other five segments such that membrane depolarization could shift the position of one helix relative to the other, thereby opening the channel. Accessory subunits may modify the function of the channel.

Therapeutic utility in recombinant materials derived from the DNA of the numerous sodium channels have been discovered. For example, U.S. Patent No. 5,132,296 discloses
20 purified Na⁺ channels that have proven useful as therapeutic and diagnostic tools.

Isoforms of sodium channels are divided into „subfamilies“. The term „isoform“ is used to mean distinct but closely related sodium channel proteins, i.e., those having an amino acid homology of approximately 60-80%. These also show strong homology in
25 functions. The term „subfamilies“ is used to mean distinct sodium channels that have an amino acid homology of approximately 80-95%. Combinations of several factors are used to determine the distinctions within a subfamily, for example, the speed of a channel, chromosomal location, expression data, homology to other channels within a species and homology to a channel of the same subfamily across species. Another consideration is an
30 affinity to tetrodotoxin („TTX“). TTX is a highly potent toxin from the puffer or fugu fish which blocks the conduction of nerve impulses along axons and in excitable membranes of nerve fibers. TTX binds to the Na⁺ channel and blocks the flow of sodium ions.

Studies using TTX as a probe have shed much light on the mechanism and structure
35 of Na⁺ channels. There are three Na⁺ channel subtypes that are defined by the affinity for TTX, which can be measured by the IC₅₀ values: TTX-sensitive Na⁺ channels (IC₅₀ ≈

1 nM), TTX-insensitive N^+ channels ($\text{IC}_{50} \approx 1\text{-}5\text{ }\mu\text{M}$), and TTX-resistant Na^+ channels ($\text{IC}_{50} \geq 100\text{ }\mu\text{M}$).

5 TTX-insensitive action potentials were first studied in rat skeletal muscle (Redfern *et al.*, Acta Physiol. Scand. 82, 70-78 (1971)). Subsequently, these action potentials were described in other mammalian tissues, including newborn mammalian skeletal muscle, mammalian cardiac muscle, mouse dorsal root ganglion cells in vitro and in culture, cultured mammalian skeletal muscle and L6 cells (Rogart, Ann. Rev. Physiol. 43, 711-725 (1980)).

10 Dorsal root ganglia neurons possess both TTX-sensitive ($\text{IC}_{50} \approx 0.3\text{ nM}$) and TTX-resistant ($\text{IC}_{50} \approx 100\text{ }\mu\text{M}$) sodium channel currents, as described in Roy *et al.*, J. Neurosci. 12, 2104-2111 (1992).

15 TTX-resistant sodium currents have also been measured in rat nodose and petrosal ganglia (Ikeda *et al.*, J. Neurophysiol. 55, 527-539 (1986) and Stea *et al.*, Neurosci. 47, 727-736 (1992)).

DESCRIPTION OF THE INVENTION

20 The present invention relates to novel sodium channel proteins. Specific embodiments include the α -subunit of such sodium channels that are TTX-sensitive.

In particular, the present invention relates to a purified and isolated DNA sequence encoding for a novel rat TTX-sensitive sodium channel protein and a splice variant thereof.
25 The term "purified and isolated DNA" refers to DNA that is essentially free, i.e. contains less than about 30%, preferably less than about 10%, and even more preferably less than about 1% of the DNA with which the DNA of interest is naturally associated. Techniques for assessing purity are well known to the art and include, for example, restriction mapping, agarose gel electrophoresis, and CsCl gradient centrifugation. The term "DNA" is meant to
30 include cDNA made by reverse transcription of mRNA or by chemical synthesis.

Specifically, the invention encompasses DNA having the engineered versions (discussed in detail below) of the nucleotide sequences set forth in SEQ ID NOS:1 and 2 designated herein as nerve sodium channel types 4 and the splice variant 4a (PN4 and
35 PN4a). These versions of the PN4 and PN4a sequence were produced by removing most of the untranslated sequences of the PN4 and PN4a cDNA and cloned into expression

vectors for functional analysis. The longer „native“ version of PN4 is shown in Fig. 3 (SEQ ID NO:7). The complete „native“ base pair sequence of PN4a has the same sequence shown in Fig. 3 and labeled rPN4 with the 30 base pair insert after position 2050. The PN4 and PN4a DNA sequences comprise cDNA sequences that encode the α -subunit of novel voltage-gated, TTX-sensitive sodium channels, specifically the amino acid sequences set forth in Figs. 1 and 2 (SEQ ID NOS: 3 and 4). DNA sequences encoding the same or allelic variant or analog sodium channel protein polypeptides of the nervous system, through use of, at least in part, degenerate codons are also contemplated by this invention. The nucleotide sequences of SEQ ID NOS:1 and 2 correspond to the cDNAs from rat. A homology search provided that the closest related sodium channel is found in the fugu (puffer fish), with 92% homology. The next closest channels are rat brain types I and II, at 87.9% and rat brain type III at 87.3%. Homology to all other known channels drops off significantly thereafter.

15 Additionally, it is believed that the novel voltage-gated, TTX-sensitive sodium channel is also expressed in tissue of other mammalian species such as humans, and that the corresponding gene is highly homologous to the rat sequence. Therefore, the invention includes cDNA encoding a novel mammalian voltage-gated, TTX-sensitive sodium channel.

20 The invention not only includes the entire protein expressed by the cDNA sequences of SEQ ID NOS:1 and 2, but also includes protein fragments. These fragments can be obtained by cleaving the full length proteins or by using smaller DNA sequences or polynucleotides to express the desired fragment. Accordingly, the invention also includes polynucleotides that can be used to make polypeptides of about 10 to 1500, preferably 10 to 100, amino acids in length. The isolation and purification of such recombinant polypeptides can be accomplished by techniques that are well known in the art, for example preparative chromatographic separations or affinity chromatography. In addition, polypeptides can also be made by synthetic means which are well known in the art.

30 In general, sodium channels comprise an α - and two J-subunits. The J-subunits may modulate the function of the channel. However, since the α -subunit is all that is required for the channel to be fully functional, expression of the cDNA in SEQ ID NOS:1 and 2, will each provide a fully functional protein. The gene encoding the J₁-subunit in nerve tissue was found to be identical to that found in rat heart, brain and skeletal muscle.

35 The cDNA of the J₁-subunit is not described herein as it is well known in the art (Isom *et al.*, Neuron 12, 1183-1194 (1994)). However, it is to be understood that by combining the

known sequence for the J₁-subunit with the α -subunit sequence described herein, one may obtain complete PN4 and PN4a rat voltage-gated, TTX-sensitive sodium channels.

Northern blot analysis indicates that PN4 and PN4a are each encoded by a
5 ~7.5 kb/9.5 kb transcript. The nucleotide sequence analysis of the PN4 cDNA identifies a 5934-base open reading frame, shown in SEQ ID NO:1, starting at base 22. The nucleotide sequence analysis of the PN4a cDNA identifies a 5964-base open reading frame, shown in SEQ ID NO:2, also starting at base 22. The deduced amino acid sequence of PN4, shown in Fig. 1 (SEQ ID NO:3), exhibits the primary structural features of an α -subunit of a
10 voltage-gated, TTX-sensitive sodium channel. Shown in Fig. 1 are the homologous domains (I-IV); the putative transmembrane segments (S1-S6); the amino acid conferring sensitivity to TTX (Δ); potential cAMP-phosphorylation site (\bullet); and potential N-glycosylation site (\blacklozenge). The deduced amino acid sequence of PN4a, shown in Fig. 2 (SEQ ID NO:4), also exhibits the primary structural features of an α -subunit of a voltage-gated, TTX-sensitive
15 sodium channel. Shown in Fig. 2 are the homologous domains (I-IV); the putative transmembrane segments (S1-S6); the amino acid conferring sensitivity to TTX (Δ); potential cAMP-phosphorylation site (\bullet); and potential N-glycosylation site (\blacklozenge).

Reverse transcription-polymerase chain reaction (degenerate oligonucleotide-primed
20 „RT-PCR“) analysis of RNA from the rat central and peripheral nervous systems, in particular from rat dorsal root ganglia („DRG“) was performed. Eight main tissue types were screened by RT-PCR for expression of the unique PN4 genes corresponding to positions 4646-5203 of SEQ ID NO:1. PN4 was present in five of the tissues studied: brain, spinal cord, DRG, nodose ganglia and superior cervical ganglia. PN4 was not present
25 in the remaining tissues studied: sciatic nerve tissue, heart or skeletal muscle tissue.

Three main tissue types were screened by RT-PCR for expression of the unique PN4a genes corresponding to positions 1947-2135 of SEQ ID NO:2. PN4a was present in two of the tissues studied: spinal cord and DRG. PN4a was not present in brain tissue.

30

The invention also pertains to the cloning and functional expression in *Xenopus* oocytes of the novel PN4 and PN4a rat TTX-sensitive sodium channels. Specifically, the α -subunit of the sodium channels was cloned and expressed. Functional expression shows that PN4 and PN4a are voltage-gated, TTX-sensitive sodium channels with properties that
35 are similar to other TTX-sensitive sodium channels.

Preferred aspects of this invention are PN4 cDNA sequences which encode for the novel mammalian TTX-sensitive sodium channel proteins that are expressed in brain, spinal cord, dorsal root ganglia, nodose ganglia and superior cervical ganglia but not in sciatic nerve, heart or skeletal muscle when assayed by the methods described herein, such as RT-PCR.

Also preferred aspects of this invention are PN4a cDNA sequences which encode for the novel mammalian TTX-sensitive sodium channel proteins that are expressed most strongly in DRG, with little expression in spinal cord and almost undetectable expression in brain when assayed by the methods described herein, such as RT-PCR.

cDNA sequences which encode for the novel PN4 TTX-sensitive sodium channel proteins that are predominantly expressed in the brain and spinal cord are also contemplated by this invention. cDNA sequences which encode for the novel PN4a TTX-sensitive sodium channel proteins that are predominantly expressed in the DRG are also contemplated by this invention.

The term „cDNA“, or complementary DNA, refers to single-stranded or double-stranded DNA sequences obtained by reverse transcription of mRNA isolated from a donor cell. For example, treatment of mRNA with a reverse transcriptase such as AMV reverse transcriptase or M-MuLV reverse transcriptase in the presence of an oligonucleotide primer will furnish an RNA-DNA duplex which can be treated with RNase H, DNA polymerase, and DNA ligase to generate double-stranded cDNA. If desired, the double-stranded cDNA can be denatured by conventional techniques such as heating to generate single-stranded cDNA. The term „cDNA“ includes cDNA that is a complementary copy of the naturally occurring mRNA as well as complementary copies of variants of the naturally occurring mRNA, that have the same biological activity. Variants would include, for example, insertions, deletions, sequences with degenerate codons and alleles. For example, PN4a is a splice variant of PN4, having a 10 amino acid insertion.

The term „crRNA“ refers to RNA that is a copy of the mRNA transcribed by a cell. crRNA corresponding to mRNA transcribed from a DNA sequence encoding the α -subunit of a novel TTX-sensitive sodium channel protein is contemplated by this invention.

The present invention also includes expression vectors comprising the DNA or the cDNA described above, host cells transformed with these expression vectors capable of producing the sodium channel of the invention, and cDNA libraries comprising such host cells.

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The term "expression vector" refers to any genetic element, e.g., a plasmid, a chromosome, a virus, behaving either as an autonomous unit of polynucleotide expression within a cell or being rendered capable of replication by insertion into a host cell chromosome, having attached to it another polynucleotide segment, so as to bring about the replication and/or expression of the attached segment. Suitable vectors include, but are not limited to, plasmids, bacteriophages and cosmids. Vectors will contain polynucleotide sequences which are necessary to effect ligation or insertion of the vector into a desired host cell and to effect the expression of the attached segment. Such sequences differ depending on the host organism, and will include promoter sequences to effect transcription, enhancer sequences to increase transcription, ribosomal binding site sequences and transcription and translation termination sequences.

15

The term "host cell" generally refers to prokaryotic or eukaryotic organisms and includes any transformable or transfectable organism which is capable of expressing a protein and can be, or has been, used as a recipient for expression vectors or other transferred DNA. Host cells can also be made to express protein by direct injection with exogenous cRNA translatable into the protein of interest. A preferred host cell is the *Xenopus* oocyte.

20

The term "transformed" refers to any known method for the insertion of foreign DNA or RNA sequences into a host prokaryotic cell. The term „transfected" refers to any known method for the insertion of foreign DNA or RNA sequences into a host eukaryotic cell. Such transformed or transfected cells include stably transformed or transfected cells in which the inserted DNA is rendered capable of replication in the host cell. They also include transiently expressing cells which express the inserted DNA or RNA for limited periods of time. The transformation or transfection procedure depends on the host cell being transformed. It can include packaging the polynucleotide in a virus as well as direct uptake of the polynucleotide, such as, for example, lipofection or microinjection. Transformation and transfection can result in incorporation of the inserted DNA into the genome of the host cell or the maintenance of the inserted DNA within the host cell in plasmid form. Methods of transformation are well known in the art and include, but are

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-8-

not limited to, viral infection, electroporation, lipofection and calcium phosphate mediated direct uptake.

5 It is to be understood that this invention is intended to include other forms of expression vectors, host cells and transformation techniques which serve equivalent functions and which become known to the art hereto.

10 The term „cDNA library“ refers to a collection of clones, usually in a bacteriophage, or less commonly in bacterial plasmids, containing cDNA copies of mRNA sequences derived from a donor cell or tissue.

15 In addition, the present invention contemplates recombinant polynucleotides, of about 15 to 20kb, preferably 10 to 15kb nucleotides in length, comprising a nucleic acid sequence derived from the DNA of the invention.

20 The term "polynucleotide" as used herein refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, this term includes double- and single-stranded DNA, as well as double- and single-stranded RNA. It also includes modified, for example, by methylation and/or by capping, and unmodified forms of the polynucleotide.

25 The term "derived from" a designated sequence, refers to a nucleic acid sequence that is comprised of a sequence of approximately at least 6-8 nucleotides, more preferably at least 10-12 nucleotides, and, even more preferably, at least 15-20 nucleotides that correspond to, i.e., are homologous or complementary to, a region of the designated sequence. The derived sequence is not necessarily physically derived from the nucleotide sequence shown, but may be derived in any manner, including for example, chemical synthesis or DNA replication or reverse transcription, which are based on the information provided by the sequences of bases in the region(s) from which the polynucleotide is
30 derived.

35 Further, the term "polynucleotide" is intended to include a recombinant polynucleotide, which is of genomic, cDNA, semisynthetic or synthetic origin which, by virtue of its origin or manipulation is not associated with all or a portion of the polynucleotide with which it is associated in nature and/or is linked to a polynucleotide other than that to which it is linked in nature.

The „native“ version of PN4, and its splice variant PN4a, partially correspond to the sodium channel NaCh6 (described by Schaller *et al.* in J. Neurosci. 15, 3231-3242 (1995)) as shown in Figs. 3 and 4. In Figs. 3 and 4, base pair and amino acid sequences of the native PN4 and PN4a sodium channel cDNA clones include untranslated sequences. The published NaCh6 sequence does appear to not correctly provide the sodium channel sequence, and the sequences for PN4 and PN4a appear to represent the authentic sodium channel sequence for the following reasons:

First, most sodium channel gene coding regions, including PN4, begin with an eleven base pair sequence consisting of an out of frame ATG, followed by five base pairs downstream, followed by the ATG initiation codon for the coding region. The DNA sequence alignment (Fig. 3) shows a two base pair deletion in NaCh6 overlapping the second ATG, so that the normally out of frame, upstream ATG becomes the NaCh6 initiation codon, leading to a two amino acid insertion. Start and stop codons are underlined and primers are denoted by dashed lines with arrows.

Examination of the DNA sequence alignment (Fig. 3) shows that the bulk of the differences (residues in bold print) between the two sequences that would strongly influence protein function consist of a series of nine single base deletions in the Interdomain I/II region. These differences lead to a very different amino acid sequence, as shown in the amino acid alignment of Fig. 4, where the differences between the two sequences are again shown in bold print. The applicants' sequencing of multiple isolates resulting from the cloning of up to 1.5kb of the Interdomain I/II region by PCR repeatedly resulted in sequences which completely agreed with PN4 or PN4a sequences.

Comparison of PN4 and PN4a sequences to other sodium channel sequences shows a high degree of homology. For example, Fig. 5 is a comparison of PN4 and PN4a with NaCh6 and rat Brain type II in this region. Whereas PN4 and BrainII share about 50% identity in the region highlighted in bold, NaCh6 is almost completely different. The differences between BrainII and PN4 are underlined.

Also PCR was employed to look specifically for NaCh6. A sense primer common to both sequences (CAATCGTGGGCGCCCTAATC, corresponding to base pair 722-742 of NaCh6 and shown by dashed lines with an arrow in Fig. 3 at bases 884-904 of PN4) was paired with gene specific antisense primers (TGCTTTCATGCACTGGAATCCCTCT,

-10-

corresponding to base pair 1194-1170 of PN4, and

TGCTTTACTGCACTGGAATCCITCG, corresponding to base pair 1029-1005 of NaCh6; sequence differences between the two primers are underlined). The antisense

primers prime at a three base pair deletion of NaCh6 relative to PN4 and overlap three

5 other sequence differences, as shown in Fig. 3. A PCR product of the expected size (about 300 base pairs) was obtained with the PN4 specific antisense primer using pBK-CMV/75-1.4 DNA (described in the description of SEQ ID NO:2) and with rat Brain and rat DRG cDNA templates. No PCR products were obtained from these templates with the NaCh6 specific primer.

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Any of the sequence differences between PN4 and NaCh6 could result in an inability of the NaCh6 gene to form a functional channel. However, some differences could be ascribed to „base calling.“ To verify the accuracy of the sequence the full length versions of PN4 and PN4a were sequenced. Of the amino acid differences between PN4 and NaCh6, 15 it appears that the profound differences in the Interdomain I/II region are responsible for the lack of success in expression of the NaCh6 gene. The nine single base deletions in this region appear to shift the reading frame (see Figs. 3 and 4), leading to a „nonsense“ peptide which lacks a number of highly conserved residues (Fig. 5) and which could sufficiently disrupt the structure of the protein to destroy its function.

20

The splice variant PN4a is similar to and occurs in a homologous position to that seen with rat type Brain1 and 1a channels (Schaller *et al.*, *J. Neurosci* 12, 1370-1381 (1992)). In each case it appears that the variants make use of the same 3' splice acceptor sites but alternative 5' sites. Rat BrainIII also has splice variants in this region, using the 25 same 3' splice site but using alternative 5' sites more 5' than the other channels. An amino acid comparison with other rat(r) and human(h) channels is shown below. Not all sodium channels have this splicing pattern.

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rPN4	GRLLE	AT.TEVE
rPN4a	GRLLE	VKIDKAAT.DS	AT.TEVE
rBRAIN1	GQLLE	VIIDKPATDDN	GTTTETE
rBRAIN1a	GQLLE	GTTTETE
rPN1	GQLLE	VIIDKATSDDS	GTTNQMR
hNE-Na	GQLLE	GTTNQIH
rBRAIN2	GQLLE	GTTTETE
rBRAIN3	GTTTETE
rCARDIAC	SYLLRP	MVLDRPP..DT	TTPSEEP

-11-

It is interesting to note that rat PN1 is similar to PN4a whereas its human homologue, the neuroendocrine channel, hNE-Na, is similar to PN4. Perhaps each of these will be found to be one of a set of splice variants. Whereas the splicing patterns of BrainI, II, and III were found not to vary across a range of tissues (Schaller *et al.*, *J. Neurosci* 12, 1370-1381 (1992)), PN4 and PN4a show dramatic abundance differences. PN4 has a gradient of expression with high expression in brain, intermediate in spinal cord and relatively the least in DRG. PN4a is very low or undetectable in brain, a minor fraction of total PN4 expression in spinal cord, and nearly as abundant as PN4 in DRG.

Many uses of the invention exist, a few of which are described below.

1. Probe for human channel.

As mentioned above, it is believed that homologs of the novel rat TTX-sensitive sodium channel described herein are also expressed in mammalian nerve tissue, in particular, human tissue. The entire cDNAs of PN4 and PN4a rat sodium channels of the present invention can be used as a probe to discover whether novel PN4 and PN4a voltage-gated, TTX-sensitive sodium channels exist in human nerve tissue and, if they do, to aid in isolating the cDNAs for the human protein.

The human homologues of the rat TTX-sensitive PN4 and PN4a channels can be cloned using a human DRG cDNA library. Human DRG are obtained at autopsy. The frozen tissue is homogenized and the RNA extracted with guanidine isothiocyanate (Chirgwin *et al.*, *Biochemistry* 18, 5294-5299 (1979)). The RNA is size-fractionated on a sucrose gradient to enrich for large mRNAs because the sodium channel α -subunits are encoded by large (7-11 kb) transcripts. Double-stranded cDNA is prepared using the SuperScript Choice cDNA kit (GIBCO BRL) with either oligo(dT) or random hexamer primers. EcoRI adapters are ligated onto the double-stranded cDNA which is then phosphorylated. The cDNA library is constructed by ligating the double-stranded cDNA into the bacteriophage-lambda ZAP II vector (Stratagene) followed by packaging into phage particles.

Phage are plated out on 150 mm plates on a lawn of XLI-Blue MRF' bacteria (Stratagene) and plaque replicas are made on Hybond N nylon membranes (Amersham). Filters are hybridized to rat PN4 and PN4a cDNA probes by standard procedures and detected by autoradiography or chemiluminescence. The signal produced by the rat PN4

-12-

and PN4a probes hybridizing to positive human clones at high stringency should be stronger than obtained with rat brain sodium channel probes hybridizing to these clones. Positive plaques are further purified by limiting dilution and re-screened by hybridization or PCR. Restriction mapping and polymerase chain reaction will identify overlapping
5 clones that can be assembled by standard techniques into the full-length human homologue of rat PN4 and PN4a. The human clone can be expressed by injecting cRNA transcribed *in vitro* from the full-length cDNA clone into *Xenopus* oocytes, or by transfecting a mammalian cell line with a vector containing the cDNA linked to a suitable promoter.

10 2. Probe for Obtaining Molecular Data.

The polynucleotides of the invention can be bound to a reporter molecule to form a polynucleotide probe useful for Northern and Southern blot analysis and *in situ* hybridization.

15 The term "reporter molecule" refers to a chemical entity capable of being detected by a suitable detection means, including, but not limited to, spectrophotometric, chemiluminescent, immunochemical, or radiochemical means. The polynucleotides of this invention can be conjugated to a reporter molecule by techniques well known in the art. Typically the reporter molecule contains a functional group suitable for attachment to or
20 incorporation into the polynucleotide. The functional groups suitable for attaching the reporter group are usually activated esters or alkylating agents. Details of techniques for attaching reporter groups are well known in the art. See, for example, Matthews et al., Anal. Biochem., 151, 205-209 (1985) and Engelhardt *et al.*, European Patent Application No. 0 302 175.

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3. Antibodies Against PN4 and PN4a.

The polypeptides of the invention are highly useful for the development of antibodies against PN4 and PN4a. Such antibodies can be used in affinity chromatography to purify recombinant sodium channel proteins or polypeptides, or they can be used as a
30 research tool. For example, antibodies bound to a reporter molecule can be used in histochemical staining techniques to identify other tissues and cell types where PN4 and PN4a are present, or they can be used to identify epitopic or functional regions of the sodium channel protein of the invention.

-13-

The antibodies can be monoclonal or polyclonal and can be prepared by techniques that are well known in the art. Polyclonal antibodies are prepared as follows: an immunogenic conjugate comprising PN4, PN4a or a fragment thereof, optionally linked to a carrier protein, is used to immunize a selected mammal such as a mouse, rabbit, goat, etc.

- 5 Serum from the immunized mammal is collected and treated according to known procedures to separate the immunoglobulin fraction.

- 10 Monoclonal antibodies are prepared by standard hybridoma cell technology based on that reported by Köhler and Milstein in Nature 256, 495-497 (1975): spleen cells are obtained from a host animal immunized with the PN4 or PN4a protein or a fragment thereof, optionally linked to a carrier. Hybrid cells are formed by fusing these spleen cells with an appropriate myeloma cell line and cultured. The antibodies produced by the hybrid cells are screened for their ability to bind to expressed PN4 or PN4a proteins.

- 15 A number of screening techniques well known in the art, such as, for example, forward or reverse enzyme-linked immunosorbent assay screening methods may be employed. The hybrid cells producing such antibodies are then subjected to recloning and high dilution conditions in order to select a hybrid cell that secretes a homogeneous population of antibodies specific to either the PN4 or PN4a protein.

20

- In addition, antibodies can be raised by cloning and expressing nucleotide sequences or mutagenized versions thereof coding at least for the amino acid sequences required for specific binding of natural antibodies, and these expressed proteins used as the immunogen. Antibodies may include the complete immunoglobulin or a fragment thereof. Antibodies
25 may be linked to a reporter group such as is described above with reference to polynucleotides.

4. Therapeutic Targets for Disorders.

- The present invention also includes the use of the novel voltage-gated, TTX-sensitive sodium channel α -subunit as a therapeutic target for compounds to treat disorders
30 of the nervous system including, but not limited to, epilepsy, stroke injury, brain injury, allodynia, hyperalgesia, diabetic neuropathy, traumatic injury and AIDS-associated neuropathy. The invention allows for the manipulation of genetic materials by recombinant technology to produce polypeptides that possess the structural and functional
35 characteristics of the novel voltage-gated, TTX-sensitive sodium channel α -subunit found in nerve tissue, particularly in sensory nerves. Site directed mutagenesis can be used to

-14-

provide such recombinant polypeptides. For example, synthetic oligonucleotides can be specifically inserted or substituted into the portion of the gene of interest to produce genes encoding for and expressing a specific mutant. Random degenerate oligonucleotides can also be inserted and phage display techniques can be used to identify and isolate polypeptides
5 possessing a functional property of interest.

5. Designing Therapeutics based on Inhibiting PN4 and PN4a and assays thereof.

The present invention is also directed to inhibiting the activity of PN4 in brain, spinal cord, DRG, nodose ganglia and superior cervical ganglia tissues. This invention is
10 also directed to inhibiting the activity of PN4a in spinal cord and DRG tissues. However, it is to be understood that further studies may reveal that PN4 and PN4a are present in other tissues, and as such, those tissues can also be targeted areas. For example, the detection of PN4 mRNA in nodose ganglia suggests that PN4 may conduct TTX-sensitive sodium currents in this and other sensory ganglia of the nervous system.

15 In addition, it has been found that proteins not normally expressed in certain tissues, are expressed in a disease state. Therefore, this invention is intended to encompass the inhibition of PN4 and PN4a in tissues and cell types where the protein is normally expressed, and in those tissues and cell types where the protein is only expressed during a
20 disease state.

The invention also pertains to an assay for inhibitors of the novel TTX-sensitive sodium channel protein comprising contacting a compound suspected of being an inhibitor with expressed sodium channel and measuring the activity of the sodium channel. The
25 compound can be a substantially pure compound of synthetic origin combined in an aqueous medium, or the compound can be a naturally occurring material such that the assay medium is an extract of biological origin, such as, for example, a plant, animal, or microbial cell extract. PN4 and PN4a activity can be measured by methods such as electrophysiology (two electrode voltage clamp or single electrode whole cell patch clamp),
30 guanidinium ion flux assays and toxin-binding assays. An "inhibitor" is defined as generally that amount that results in greater than 50% decrease in PN4 or PN4a activity, preferably greater than 70% decrease in PN4 or PN4a activity, more preferably, greater than 90% decrease in PN4 or PN4a activity.

35 6. Designing and Screening for Additional Therapeutics.

-15-

Another significant characteristic of PN4 is that it is TTX-sensitive. It is believed that TTX-sensitive sodium channels play a key role in transmitting nerve impulses relating to sensory inputs such as pain and pressure. This will also facilitate the design of therapeutics that can be targeted to a specific area such as nerve tissue.

5

Additionally, the recombinant protein of the present invention can be used to screen for potential therapeutics that have the ability to inhibit the sodium channel of interest. In particular, it would be useful to inhibit selectively the function of sodium channels in nerve tissues responsible for transmitting pain and pressure signals without simultaneously affecting the function of sodium channels in other tissues such as heart and muscle. Such selectivity would allow for the treatment of pain without causing side effects due to cardiac or neuromuscular complications. Therefore, it would be useful to have DNA sequences coding for sodium channels that are selectively expressed in nerve tissue.

10

7. Pain Reliever.

Sodium channels in nerve tissue play a large role in the transmission of nerve impulses, and therefore are instrumental in understanding neuropathic pain transmission. Neuropathic pain falls into two categories: allodynia, where a normally non-painful stimulus becomes painful, and hyperalgesia, where a usually normal painful stimulus becomes extremely painful. The ability to inhibit the activity of these sodium channels, i.e., reduce the conduction of nerve impulses, will affect the nerve's ability to transmit pain. Selective inhibition of sodium channels in sensory neurons such as dorsal root ganglia will allow the blockage of pain impulses without complicating side effects caused by inhibition of sodium channels in other tissues such as brain and heart. In addition, certain diseases are caused by sodium channels that produce impulses at an extremely high frequency. The ability to reduce the activity of the channel can then eliminate or alleviate the disease. Accordingly, potential therapeutic compounds can be screened by methods well known in the art, to discover whether they can inhibit the activity of the recombinant sodium channel of the invention. See Barram *et al.*, Naun-Schmiedeberg's archives of Pharmacology, 347, 125-132 (1993) and McNeal *et al.*, J. Med. Chem., 28, 381-388 (1985). For similar studies with the acetyl choline receptor, see, Claudio *et al.*, Science 238, 1688-1694 (1987).

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Accordingly, the present invention encompasses a method of alleviating pain by inhibiting the activity of the novel TTX-sensitive sodium channel comprising administering a therapeutically effective amount of a compound having an IC_{50} in the range of 0.1-50 nM, preferably within the range of 1-25 nM. and most preferably within the range of 1-5 nM.

-16-

Potential therapeutic compounds are identified based on their ability to inhibit the activity of PN4 and PN4a. Therefore, the aforementioned assay can be used to identify compounds having a therapeutically effective IC_{50} .

- 5 The term „ IC_{50} “ refers to the concentration of a compound that is required to inhibit by 50% the activity of expressed PN4 or PN4a when activity is measured by electrophysiology, flux assays and toxin-binding assays, as mentioned above.

- 10 The basic molecular biology techniques employed in accomplishing features of this invention, such as RNA, DNA and plasmid isolation, restriction enzyme digestion, preparation and probing of a cDNA library, sequencing clones, constructing expression vectors, transforming cells, maintaining and growing cell cultures and other general techniques are well known in the art, and descriptions of such techniques can be found in general laboratory manuals such as *Molecular Cloning: A Laboratory Manual* by Sambrook, 15 *et al.* (Cold Spring Harbor Laboratory Press, 2nd edition, 1989). Accordingly, the following examples are merely illustrative of the techniques by which the invention can be practiced.

20 BRIEF DESCRIPTION OF THE SEQ ID'S AND FIGURES

- 25 SEQ ID NO:1 depicts an engineered version of the nucleotide cDNA sequence encoding the rat TTX-sensitive peripheral nerve sodium channel type 4 („PN4“). This version lacks most of the untranslated sequences, thereby comprising a 5934-base open reading frame, from nucleotide residue 22 of the XhoI-HindIII clone, the start site of translation, and ending at residue 5956.

- 30 SEQ ID NO:2 depicts an engineered version of the nucleotide cDNA sequence encoding the rat TTX-sensitive peripheral nerve sodium channel type 4a („PN4a“). This version lacks most of the untranslated sequences, thereby comprising a 5964-base open reading frame, beginning at nucleotide residue 22 of the XhoI-HindIII clone, the start site of translation, and ending at residue 5986. The 30 base pair insert is found at positions 2014-2043.

- 35 Fig. 1 (SEQ ID NO:3) depicts the deduced amino acid sequence of PN4, represented in the single-letter amino acid code. Shown in Fig. 1 are the homologous domains (I-IV); the

-17-

putative transmembrane segments (S1-S6); the amino acid conferring sensitivity to TTX (Δ); potential cAMP-phosphorylation site (\bullet); and potential N-glycosylation site (\blacklozenge).

5 Fig. 2 (SEQ ID NO:4) depicts the deduced amino acid sequence of PN4a, represented in the single-letter amino acid code. Shown in Fig. 2 are the homologous domains (I-IV); the putative transmembrane segments (S1-S6); the amino acid conferring sensitivity to TTX (Δ); potential cAMP-phosphorylation site (\bullet); and potential N-glycosylation site (\blacklozenge).

10 Fig. 3 aligns the base pair sequences of the NaCh6 and the „native“ version of the PN4 sodium channel cDNA clones (SEQ ID NO:7), including untranslated sequences, depicting the differences in bold. Start and stop codons are underlined and primers are denoted by dashed lines with arrows.

15 Fig. 4 aligns the amino acid sequences of the PN4a, PN4 and NaCh6 sodium channel cDNA clones of Fig. 3, depicting the differences in bold.

20 Fig. 5 is a comparison of the conserved region Interdomain I/II between PN4a, PN4, NaCh6 and BrainII sodium channels. Differences between PN4 (and PN4a) and NaCh6 are shown in bold type and differences between BrainII and PN4 are underlined.

SEQ ID NO:5 depicts the 696 nucleotide cDNA sequence encoding the novel probe CNaD4-2 used to identify the novel sodium channels of the invention.

25 SEQ ID NO:6 depicts the deduced amino acid sequence of probe CNaD4-2, represented in the single-letter amino acid code.

Fig. 6 depicts the cloning map of PN4 and PN4a.

30 Fig. 7 shows the properties of currents produced in *Xenopus* oocytes by injection of PN4 cRNA. Fig. 7a shows the current produced by sodium channels expressed in oocyte, Fig. 7b shows the current-voltage relationship.

35 Fig. 8a and 8b show steady state inactivation of sodium currents produced by PN4 in *Xenopus* oocytes.

-18-

Fig. 9 demonstrates the effects of the β_1 and β_2 subunits upon PN4 function in *Xenopus* oocytes. Fig. 9a shows currents produced when the PN4 I subunit is injected alone; Fig. 9b is with the β_1 subunit; Fig. 9c is with the β_2 subunit; and Fig. 9d is with both the β_1 and β_2 subunits.

5

Abbreviations

	BSA	bovine serum albumin
10	Denhardt's solution	0.02% BSA, 0.02% polyvinyl-pyrrolidone, 0.02% Ficoll (0.1 g BSA, 0.1 g Ficoll and 0.1 g polyvinylpyrrolidone per 500 ml)
	DRG	dorsal root ganglia
	EDTA	Ethylenediaminetetraacetic acid, tetrasodium salt
15	MEN	20 mM MOPS, 1 mM EDTA, 5 mM sodium acetate, pH 7.0
	MOPS	3-(N-morpholino)propanesulfonic acid (Sigma Chemical Company)
	PN3	peripheral nerve sodium channel type 3
	PNS	peripheral nervous system
20	SDS	sodium dodecyl sulfate
	SSC	150 mM NaCl, 15 mM sodium citrate, pH 7.0
	SSPE	80 mM NaCl, 10 mM sodium phosphate, 1 mM ethylenediaminetetraacetate, pH 8.0
	TEV	two electrode voltage clamp
25	TTX	tetrodotoxin (Sigma Chemical Company)
	UTR	untranslated region

EXAMPLES

30 Each step employed in obtaining the DNA of the novel sodium channel of the invention is described in the detailed examples below. The following is an overview of the steps. Example 1 describes how a novel probe, CNaD4-2, was obtained by designing primers based on known sodium channels. Example 2 describes the construction and screening of a cDNA library with CNaD4-2 to obtain the 3' end of the novel sodium
35 channel of the invention. Then, a known primer was employed to obtain the 5' end of the DNA of the invention. Example 3 describes how RT-PCR was employed to span the gap,

-19-

between the 3' and 5' ends obtained from the cDNA library. This resulted in a 798 base pair sequence and a splice variant thereof, having a 828 base pair sequence. Example 4 describes assembling the clones into two full-length cDNA clones in expression vectors. The cloning map is illustrated in Fig. 6. Example 5 discusses the tissue distribution and localization accomplished by RT-PCR. Example 6 discusses the northern analysis of mRNA. Example 7 discloses obtaining expression data from *Xenopus* oocytes, and localization by RT-PCR.

Materials

The plasmid pBK-CMV was obtained from Stratagene (La Jolla, CA); plasmid Litmus 29 was obtained from New England Biolabs (Beverly, MA); the oocyte expression vector plasmid pBSTAcIIr was constructed from pBSTA (described by Goldin *et al.*, in Methods in Enzymology (Rudy & Iverson, eds.) 207, 279-297) by insertion of a synthetic oligonucleotide linker; the mammalian cell expression vector plasmid pCI-neo was obtained from Promega (Madison, WI); plasmid pCRII was obtained from Invitrogen, San Diego, CA. Competent *E. Coli* cell lines STBL2™ and SURE® were obtained from GIBCO/BRL and Stratagene, respectively.

EXAMPLE 1: Identification of a novel channel fragment

A novel probe used to identify the novel sodium channels was obtained as follows. Degenerate oligonucleotide primers were designed based on the homologies between known sodium channels in domain IV and used to perform RT-PCR on RNA isolated from rat DRG. The domain IV PCR products were cloned into pCRII, transformed into *E. coli* and single colonies isolated. DNA sequence of the inserts of several of these colonies was obtained, including the following novel sequence from clone pCRII/CNaD4-2 of SEQ ID NO:5, identified as CNaD4-2. SEQ ID NO:6 depicts the deduced amino acid sequence of probe CNaD4-2, represented in the single-letter amino acid code.

CNaD4-2 can be made with standard PCR techniques.

EXAMPLE 2: Construction and screening of cDNA library from rat DRG with probe CNaD4-2

EcoRI-adapted cDNA was prepared from normal adult male Sprague-Dawley rat DRG poly(A)+ RNA using the SuperScript Choice System (GIBCO BRL). cDNA (>4kb) was selected by sucrose gradient fractionation as described by Kieffer (Gene 109, 115-119 (1991)). The cDNA was then ligated into the Zap Express vector (Stratagene), and

-20-

packaged with the Gigapack II XL lambda packaging extract (Stratagene). Plate lysates were prepared and screened by PCR using CNaD4-2 specific primers (ACACTCAGAGCAAGCAGATGG and TCCCTGGGTGCTCTTTGTCCA, corresponding to bases 32 to 52 and 569 to 589 of SEQ ID NO:5, respectively). Phage from one positive lysate were screened by filter hybridization with a ³²P-labeled probe (the 700 base pair EcoRI insert from CNaD4-2). Filters were hybridized in 50% formamide, 5X SSPE, 5X Denhardt's solution, 0.5% SDS, 250µg/ml sheared, denatured salmon sperm DNA, and 50mM sodium phosphate at 42°C and washed in 0.5X SSC, 0.1% SDS at 50°C. Positive clones were excised in vivo into pBK-CMV using the ExAssist/XLOLR system (Stratagene).

Approximately 95% of these clones contained sodium channel sequence under standard screening stringency conditions. The number of clones that are retrieved that contain sodium channel sequence can be increased with increased stringency conditions and careful analysis and interpretation of data. It is well known in the art when screening for a particular type of DNA sequence, other types of DNA sequences will also be hybridized, depending on the specificity of the probe. Here, with the careful designed probe of the invention, the approximate 95% „hit“ rate, makes this fragment an exceptionally good sodium channel probe.

One of these clones, pBK-CMV/PN4.10-1, contained sequence of the CNaD4-2 channel from domain II through the 3' UTR. The position of the pBK-CMV/PN4.10-1 fragment in the PN4 and PN4a cloning map is shown in Fig. 6. In Fig. 6, ATG is the start codon, TAC is the stop codon and ∇ is the position of the PN4a splice insert.

A degenerate primer designed for sodium channels in domain I (ACCAACTG[T/C]GT[G/A]TT[T/C]ATGAC) was paired with a PN4 specific primer from the domain II region of pBK-CMV/PN4.10-1 (CAGCAGCTACAGTGGCTACA). These primers amplified a ca 1.5kb fragment from rat brain and from rat DRG which was shown by sequencing to represent much of the 5' end of PN4, thus verifying that the primers would work for screening the library. The primers were then used to screen plate lysates of the DRG cDNA library by PCR. Positive lysates were plated and individual plaques picked and screened by PCR using the same primers. Positive clones were excised in vivo into pBK-CMV using the ExAssist/XLOLR system. One of these, pBK-CMV/75-1.4, was found to contain PN4 sequence from the 5' UTR to the interdomain I/II region, but

not to domain II, possibly due to rearrangement during the excision process. The position of the pBK-CMV/75-1.4 fragment in the PN4 and PN4a cloning map is shown in Fig. 6.

EXAMPLE 3: Cloning the interdomain I/II region

5 The gap between pBK-CMV/75-1.4 and pBK-CMV/PN4.10-1 was cloned by RT-PCR on rat DRG and brain total RNA using specific primers: AAAGAGGCCGAGTTCAAGGC (a base pair sequence of pBK-CMV/75-1.4) and TGTCTTCCGTCCGTAGG (a base pair sequence of pBK-CMV/PN4.10-1). PCR products were cloned into plasmid pCRII and sequenced. Two distinct sequences, FA-2
10 and FA-7 (see Fig. 6), were cloned from DRG. These were found to be identical except for the presence of a 30 base pair insert (found at base pairs 2014-2043 in SEQ ID NO:2, and depicted by an upside triangle at the position of insertion in FA-7, FJ-13 and PN4a in Fig. 6), with sequence identity to pBK-CMV/75-1.4 and pBK-CMV/PN4.10-1 in the regions where they overlap. RT-PCR on rat brain RNA yielded only clones which lacked the 30
15 base pair insert. This insert is homologous to a splice variant of the NaChI channel (NaChIa) and likely results from alternative 5' splice site usage (Schaller *et al.*, *J. Neurosci* 12, 1370-1381 (1992)).

Additional RT-PCR was performed on rat DRG RNA using primers
20 TTCATGGGGAACCTTCGAAAC (a base pair sequence of pBK-CMV/75-1.4) and GAACGATGCAGATGGTGATGGCTAA (a base pair sequence of pBK-CMV/PN4.10-1). The 1.5kb PCR product was cloned into pCRII; six out of twenty isolates were positive for the 30 base pair insert variant by PCR. The sequence obtained for one of these, FJ-13, position shown in Fig. 6, was identical to that expected from the sequences of
25 pBK-CMV/75-1.4, FA-7, and pBK-CMV/PN4.10-1, thus confirming that these clones all originated from the same transcript.

EXAMPLE 4: Assembly of full-length PN4 clones in expression vectors

Unsuccessful attempts have been made to create and stabilize full-length sodium
30 channel cDNA sequences. In US Patent No. 5,380,836, the cDNA sequence for a rat cardiac sodium channel protein was contained in three separate plasmids. In order to create full-length functional PN4 genes, the 5' end was modified: suitable restriction sites were added and the upstream out-of-frame initiation codon was removed. The modified pBK-CMV/75-1.4 and FA-2 sequences were fused together, then combined with the remaining
35 portion of PN4 from pBK-CMV/PN4.10-1 in suitable expression vectors. PCR was employed to assemble the 5' portion of PN4 from the initiation codon to domain II. A

-22-

1.43kb PCR fragment was generated from pBK-CMV/75-1.4 using the following primers: (1) GAAGCTCGAGCCCGGGCAAGAGAAGATGGCAGCGCGG (Xho-I Srf-I restriction sites underlined, initiation codon in bold, PN4 homology in italics, a base pair sequence of pBK-CMV/75-1.4) and primer (2) CTCGGAGAGCCTACCCCATC (a base pair sequence of pBK-CMV/75-1.4, and a base pair sequence of FA-2). A 0.69kb PCR fragment was generated from FA-2 using primer (3) AGAAGGGGAAGATGGGGTAGG (a base pair sequence of FA-2, and a base pair sequence of pBK-CMV/75-1.4) and primer (4) ATTCTGTCCTTCCGTCCGTAG (a base pair sequence of FA-2, and a base pair sequence of pBK-CMV/PN4.10-1). These fragments were gel purified and then a small fraction of each was combined as template in a further PCR reaction using primers (1) and (4). The fragments share a 31 base pair region of overlap at their 3' and 5' ends respectively, and therefore can act as primers to fuse the two fragments together (Horton *et al.*, *Gene* 77, 61-68 (1991)). The 2.1kb PCR product was cloned into pCRII and several isolates were sequenced, one of which, FD-8, had the expected sequence. The position of FD-8 in the PN4 and PN4a clones is shown in the cloning map of Fig. 6.

To facilitate cloning into pBSTA and pCI-neo, it was determined to introduce an XbaI site at the 3' end. To accomplish this, the PN4 domain II to 3' UTR region was subcloned from pBK-CMV/PN4.10-1 from the EcoRI site of the vector to the HindIII site 14 base pairs from the PN4 stop codon into EcoRI plus HindIII digested Litmus 29. The resulting clone was labeled FC-1. The position of FC-1 in the PN4 and PN4a clones is shown in the cloning map of Fig. 6.

To assemble the full length PN4, the 5' portion was subcloned from FD-8 as a 2.0kb Xho I-Eco NI fragment together with the 3' portion from FC-1 as a 4.0kb Eco NI-Xba-I fragment into Xho-I plus Xba-I digested pBSTAcIIr. One of the resulting isolates was found to have the correct sequence and was named pBSTAcIIr_PN4(FU-7A).

The splice variant, PN4a, was assembled by replacing the 1.3kb Sph I - Acc I region of pBSTAcIIr_PN4(FU-7A) with the corresponding fragment from FJ-13, to form pBSTAcIIr_PN4a(FZA-3), and confirmed by DNA sequencing.

PN4 and PN4a were recloned into pCI-neo as 6.0kb Xho-I to Xba-I fragments to form pCI-neo-PN4(GAII-1) and pCI-neo-PN4a(GCII-2), respectively, and confirmed by DNA sequencing. The sequences of the coding regions as cloned in the oocyte and

mammalian cell expression vectors of PN4 and PN4a are SEQ ID NO:1 and SEQ ID NO:2, respectively.

Growth of fragments of PN4 or PN4a was accomplished under standard conditions, however growth of plasmids containing full length constructs of PN4 and PN4a (in pCIneo or pBSTAcIIr) could not be accomplished without use of special growth media, conditions, and *E. coli* strains. The following proved to be optimal: (1) use of *E. coli* STBL2™ for primary transformation following ligation reactions; for large scale culturing the primary transformants in STBL2™ cells were used, but secondary transformants in SURE® cells were used later if necessary. These *E. coli* strains have altered genotypes which allow the stable propagation of plasmids containing unstable inserts. (2) Solid media was 1/2x FM (see below) plus either 1x YENB (Bacto Yeast Extract, 0.75%, Bacto Nutrient Broth, 0.8%; Sharma and Schimke, Biotechniques 20, 42-44 (1996)), 1x YET (Bacto Yeast Extract, 0.75%, Bacto Tryptone, 0.8%), or 1x LB (Tryptone, 1%, Yeast Extract, 0.5%, NaCl, 0.5%), plus 15g/L agar. (3) Liquid media optimally was 1x FM plus 1/2x LB. (4) Carbenicillin, 100µg/ml, was used for all media, as it is metabolized less rapidly than ampicillin. However, carbenicillin may be used within the range of 50-200 µg/ml; and more preferably within the range of 75-125 µg/ml. (5) Temperature for growth should be no greater than 30°C, usually 28°C; this necessitated longer growth periods than normally employed, from 36 to 48 hours.

The recipe for 2x Freezing Medium (2xFM) is K₂HPO₄, 12.6g; Na₃Citrate, 0.9g; MgSO₄·7H₂O, 0.18g; (NH₄)₂SO₄, 1.8g; KH₂PO₄, 3.6g; Glycerol, 88g; H₂O, qs to 1L.

2xFM and the remaining media components are prepared separately, sterilized by autoclaving, cooled to at least 60°C, and added together to form the final medium. Carbenicillin is prepared at 25mg/ml H₂O and sterilized by filtration. 2xFM was first described for preparation of frozen stocks of bacterial cells (Practical Methods in Molecular Biology, Schleif, R.F. and Wensink, P.C., Springer-Verlag, New York (1981) pp201-202).

EXAMPLE 5: Tissue distribution by RT-PCR

Brain, spinal cord, DRG, nodose ganglia, superior cervical ganglia, sciatic nerve, heart and skeletal muscle tissue were isolated from anesthetized, normal adult male Sprague-Dawley rats and were stored at -80°C. RNA was isolated from each tissue using RNazol (Tel-Test, Inc.). Random-primed cDNA was reverse transcribed from 500ng of

-24-

RNA from each tissue. The CNaD4-2 specific primers
 ACACTCAGAGCAAGCAGATGG and TCCCTGGGTGCTCTTTGTCCA (see above)
 defined a 558 base pair amplicon and would not discriminate between PN4 and PN4a.
 Thermal cycler parameters were 30 s/94°C, 30 s/64°C, 1 min/72°C (24 cycles (confirmation
 5 experiment: 34 cycles)); 30 s/94°C, 30 s/64°C, 5 min/72°C (1 cycle). A positive control
 (pCRII/CNaD4-2) and a no-template control were also included. cDNA from each tissue
 was also PCR amplified using primers specific for glyceraldehyde-3-phosphate
 dehydrogenase to demonstrate template viability, as described by Tso *et al.*, Nucleic Acid
 Res. 13, 2485-2502 (1985).

10

Tissue distribution profile of PN4 by analysis of RNA from selected rat tissues by
 RT-PCR was as follows:

	<u>Tissue</u>	<u>RT-PCR (35 cycles)</u>
15	Brain	+++++
	Spinal cord	+++
	DRG	++
	Nodose ganglia	++
	Superior cervical ganglia	+
20	Sciatic nerve	-
	Heart	-
	Skeletal muscle	-

PN4 was also detected after only 25 cycles (24 + 1) in the same five tissues as
 25 above in the same relative abundance.

Since PN4 differs from PN4a by only 30 base pairs, a new sense primer,
 GGTGGACTGCAACGGCGTA (corresponding to the same base pair sequences of FA-2
 and FA-7), was employed. RT-PCR using this primer together with primer
 30 ATTCTGTCCTTCCGTCCGTAG (primer 4 above) gave amplicons of 159 base pairs
 from PN4 and 189 base pairs from PN4a. Thermal cycler parameters were 1 min/95°C;
 20sec/94°C, 30 sec/60°C, 1 min/72°C, 8 cycles; 20sec/94°C, 30 sec/58°C, 1 min/72°C, 27
 cycles; 3 min/72°C. PN4a was nearly as abundant as PN4 in DRG, much less abundant
 than PN4 in spinal cord, and almost undetectable in brain. This correlates well with cloning
 35 data; based on sequenced, cloned RT-PCR fragments which included the 30 base pair insert

-25-

region, PN4a was found in 40% of isolates from DRG (9/24), but not found from brain (0/4).

5	<u>Tissue</u>	<u>RT-PCR (35 cycles)</u>	
		<u>PN4</u>	<u>PN4a</u>
	Brain	+++++	(+ / -)
	Spinal cord	+++	+
	DRG	++	++

10 EXAMPLE 6: Northern Analysis of mRNA from rat DRG

Lumbar DRG #4 and #5 (L4 and L5), brain and spinal cord were removed from anesthetized adult male Sprague-Dawley rats under a dissecting microscope. The tissues were frozen in dry ice and homogenized with a Polytron homogenizer; the RNA was extracted by the guanidine isothiocyanate procedure (Chomczynski *et al.*, Anal.

15 Biochemistry 162, 156-159 (1987)). Total RNA (5 µg of each sample) was dissolved in MEN buffer containing 50% formamide, 6.6% formaldehyde and denatured at 65°C for 5-10 minutes. The RNA was electrophoresed through a 0.8% agarose gel containing 8.3% formaldehyde in MEN buffer. The electrode buffer was MEN buffer containing 3.7% formaldehyde; the gel was run at 50 V for 12-18 hour.

20

After electrophoresis, the gel was rinsed in 2xSSC and the RNA was transferred to a Duralose membrane (Stratagene) with 20xSSC by capillary action; the membrane was baked under vacuum at 80°C for 1 hour. The membrane was prehybridized in 50% formamide, 5xSSC, 50 mM sodium phosphate, pH 7.1, 1x Denhardt's solution, 0.5% SDS, and sheared, 25 heat-denatured salmon sperm DNA (1 mg/ml) for 16 hour at 42°C. The membrane was hybridized in 50% formamide, 5xSSC, 50 mM sodium phosphate, pH 7.1, 1x Denhardt's solution, 0.5% SDS, and sheared, heat-denatured salmon sperm DNA (200 µg/ml) with a ³²P- labeled cRNA probe (ca. 1-3x10⁶ cpm/ml). The probe was the cloned fragment, CNaD4-2, which contains the Domain 4 sequence of PN4 sodium channel α-subunit 30 sequence. The probe was hybridized for 18 hour at 42°C. The cRNA probe was synthesized by excising and subcloning the fragment into pBluescript KS+ vector, purchased from Stratagene. The cRNA was transcribed *in vitro* using T3 RNA polymerase, purchased from Promega, after linearizing the plasmid with XbaI, purchased from Boehringer Mannheim. Protocols for each procedure mentioned above can be found in 35 Molecular Cloning: A Laboratory Manual by Sambrook *et al.* (Cold Spring Harbor Laboratory Press, 2nd edition, 1989).

-26-

The membrane was washed three times with 2xSSC, 0.1% SDS at room temperature for 20 minutes and then washed once with 0.1xSSC, 0.1% SDS at 68°C for 30 minutes.

5 The filter was exposed against Kodak X-omat AR film at -80°C with intensifying screens for up to two weeks.

Size markers, including ribosomal 18S and 28S RNAs and RNA markers (GIBCO BRL), were run in parallel lanes of the gel. Their positions were determined by staining the excised lane with ethidium bromide (0.5 µg/ml) followed by photography under UV light.

10 The CNaD4-2 probe hybridized to RNA from the brain, cerebellum, dorsal and ventral horn of the spinal cord with sizes of 11 kb, 9.5 kb, 7.5 kb, and 6.5 kb, estimated on the basis of their positions relative to the standards.

15 Bands of the same size were detected in a blot containing total RNA from DRG from neuropathic pain model. However, no signal was detected with RNA from naive DRG.

PN4 constitutes a subfamily of novel sodium channel genes; these genes are different from those detectable with other probes (e.g., PEA8 and PN3 probes), as
20 discussed in copending application no. 08/511,828. Sequence comparison of PN4 with NaCh6 (mRNA size = 9.5kb) (Schaller *et al.*, J. Neurosci. 15, 3231-3242 (1995)) and cardiac-specific sodium channel for which only a partial sequence is available (mRNA size = 7kb) (Sills *et al.*, J. Clin. Invest. 84, 331-336 (1989)) indicates that these genes share a
25 higher homology among themselves than with members of other sodium channel subfamilies such as the brain-type sodium channels, TTX-insensitive cardiac sodium channel and the TTX-resistant PN3 sodium channel.

Semiquantitation of the signal intensity of the various bands detected in the blot containing RNAs from the neuropathic pain model indicated that the level of 7.5kb
30 transcript was upregulated ~35 fold as compared with the DRG from the sham operated side on day 1 after the surgery, wherein the sciatic nerve was ligated with four loose ligatures causing a constriction injury. None of the other transcripts detected by the CNaD4-2 probe was regulated so dramatically. By day 2, the regulation was reduced to ~5
35 fold as compared with the sham operated side. The experiment was performed with DRG pooled from 6 rats.

-27-

This experimental data suggests that PN4, or its splice variant, PN4a, is involved in the pathophysiology of neuropathic pain.

EXAMPLE 7: Expression of full length clone in *Xenopus* oocytes

- 5 cRNA was prepared from PN4 subcloned into pBSTA using a T7 in vitro transcription kit (Ambion, mMessage mMachine) and was injected into stage V and VI *Xenopus* oocytes using a Nanojector (Drummond), as described in Goldin, supra. After 1.5 days at 20°C, the oocytes were impaled with agarose-cushion electrodes (0.3-0.8 MOhm) and voltage-clamped with a Geneclamp 500 amplifier (Axon Instruments) in TEV mode.
- 10 See Schreibmayer *et al.*, Pflugers Arch. 426, 453-458 (1994).

- Stimulation and recording were controlled by a computer running pClamp (Axon Instruments) (Kegel *et al.* J. Neurosci. Meth. 12, 317-330 (1982)). Oocytes were perfused with a solution containing: 81 mM NaCl, 2 mM KCl, 1 mM MgCl₂, 0.3 mM
- 15 CaCl₂, 20 mM Hepes-NaOH, pH 7.5. The data collected is shown in the Figs. 7-9 and described on the following pages.

- Fig. 7a shows the currents produced from a PN4 sodium channel expressed in a *Xenopus* oocyte using the Geneclamp P/-4 leak subtraction, filtered at 5 kHz with a 4-pole
- 20 Bessel filter, and sampled at 50 kHz. Test pulses for TTX c/r were to 0 mV for 5 ms at 0.033 Hz. The x-axis denotes time in milliseconds.

- Fig. 7b illustrates the voltage to current relationship of a PN4 sodium channel expressed in a *Xenopus* oocyte.
- 25

Fig. 8a and b show steady state inactivation of sodium currents produced by PN4 in *Xenopus* oocytes. In Fig. 8a the x-axis denotes time in milliseconds. In Fig. 8b the x-axis is conditioning potential in millivolts and the y-axis is current in TA.

- 30 Fig. 9 demonstrates the effects of the β_1 and β_2 subunits upon PN4 function in *Xenopus* oocytes. Shown are currents produced when the PN4 I subunit is injected (a) alone; (b) with the β_1 subunit; (c) with the β_2 subunit; and (d) with both the β_1 and β_2 subunits. The x-axis in each of these figures denotes time in milliseconds. As these figures show, the inactivation kinetics of a functionally active PN4 channel are accelerated by the
- 35 β_1 subunit. No obvious effects are seen with the β_2 subunit.

-28-

As is seen in Fig. 7a and b expression of PN4 and PN4a produced an inward current with slow inactivation kinetics, similar to that of the rBIIa (Patton *et al.*, Neuron 7, 637-647 (1991)) and rSkM1 α -subunits expressed in the absence of the β_1 -subunit. In the expression of PN4, 0.2 ng of cRNA gave $1.4 \pm 0.19 \mu\text{A}$ ($n = 9$). In the expression of PN4a, 0.1 ng gave $1.8 \pm 0.23 \mu\text{A}$ ($n = 6$). Co-injection of rCN β_1 (1 ng/oocyte) with PN4 cRNA accelerated inactivation kinetics of the channel, as seen in Fig. 9a and b. This agreed with data obtained with rBIIa and hSCN β_1 .

For steady state inactivation, 10 second prepulses were used. In the steady state inactivation of PN4, $V_{1/2} = -70.7 \pm 0.71 \text{ mV}$, $k = 5.5 \pm 0.55 \text{ mV}$ ($n = 3$). In the steady state inactivation of PN4a, $V_{1/2} = -73.3 \pm 0.97 \text{ mV}$, $k = 5.5 \pm 0.28 \text{ mV}$ ($n = 4$). Leak currents were measured during long pulses to -100 mV and -120 mV, and the test currents corrected assuming that the leak currents had a linear current-voltage relationship. An inactivation of -70 mV is similar to most sodium channels.

TEVC activation data for PN4a was $V_{1/2} = -23 \pm 2.7 \text{ mV}$, $k = -6.4 \pm 0.75 \text{ mV}$ ($n = 3$).

TTX IC 50 was found to be $1.0 \pm 0.60 \text{ nM}$ ($n = 2$).

Sodium channels are distinctively sensitive or insensitive to neurotoxins such as TTX. The TTX-sensitive brain and skeletal muscle sodium channels are blocked by nanomolar TTX concentrations, whereas the TTX-insensitive cardiac sodium channels are blocked by micromolar TTX concentrations. In rat heart sodium channel 1 (rh1), Cys³⁷⁴ is a critical determinant of TTX-insensitivity, as shown in Satin *et al.*, Science 256, 1202-1205(1992); in the TTX-sensitive rBI, rBII, rBIII, and rSkM1, the corresponding residue is either Phe or Tyr. When expressed in *Xenopus* oocytes, the PN4 sodium current is sensitive to TTX ($\text{IC}_{50} \geq 1 \text{ nM}$).

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

-29-

SEQUENCE LISTING

- (1) GENERAL INFORMATION:
- (i) APPLICANT:
- 5 (A) NAME: F. HOFFMANN-LA ROCHE AG
(B) STREET: Grenzacherstrasse 124
(C) CITY: Basle
(D) STATE: BS
(E) COUNTRY: Switzerland
10 (F) POSTAL CODE (ZIP): CH-4002
(G) TELEPHONE: 061 - 688 42 56
(H) TELEFAX: 061 - 688 13 95
(I) TELEX: 962292/965542 hlr ch
- 15 (ii) TITLE OF INVENTION: Novel Cloned Tetrodotoxin-Sensitive Sodium
Channel I-Subunit And A Splice Variant Thereof
- (iii) NUMBER OF SEQUENCES: 7
- (iv) COMPUTER READABLE FORM:
- 20 (A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: Apple Macintosh
(C) OPERATING SYSTEM: System 7.1 (Macintosh)
(D) SOFTWARE: Word 5.0
- (2) INFORMATION FOR SEQ ID NO:1:
- 25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5977 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- 30 (ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
- 35 (A) ORGANISM: rat
(F) TISSUE TYPE: Dorsal root ganglia
(G) CELL TYPE: Peripheral nerve

-30-

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-31-

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-32-

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(3) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6007 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

-33-

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

5 (vi) ORIGINAL SOURCE:

(A) ORGANISM: rat

(F) TISSUE TYPE: Dorsal root ganglia

(G) CELL TYPE: Peripheral nerve

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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3901 TACTCGGAAC TAGGTGCCAT AAAGTCCCTT AGGACCCTAA GAGCTTTGAG ACCCTTAAGA
3961 GCCTTATCAC GATTTGAAGG GATGAGGGTG GTGGTGAATG CCTTGGTGGG CGCCATCCCC
5 4021 TCCATCATGA ATGTGCTGCT GGTGTGTCTC ATCTTCTGGC TGATTTTCAG CATCATGGGA
4081 GTTAACCTGT TTGCGGGGAA ATACCACTAC TGCTTTAATG AGACTTCTGA AATCCGGTTC
4141 GAAATCGATA TTGTCAACAA TAAAACGGAC TGTGAGAAGC TCATGGAGGG CAACAGCACG
4201 GAGATCCGAT GGAAGAATGT CAAGATCAAC TTTGACAATG TCGGAGCAGG GTACCTGGCC
4261 CTTCTTCAAG TGGCAACCTT CAAAGGCTGG ATGGACATCA TGTATGCGGC TGTAGATTCC
10 4321 CGAAAGCCAG ACGAGCAGCC TGACTACGAG GGCAACATCT ACATGTACAT CTACTTCGTC
4381 ATCTTCATCA TCTTCGGCTC CTTCTTCAAC CTCAACCTGT TCATCGGTGT CATCATCGAC
4441 AACTTCAACC AGCAGAAGAA AAAGTTTGA GGTCAAGACA TCTTCATGAC AGAGGAACAG
4501 AAGAAGTACT ACAATGCCAT GAAAAAGCTG GGCTCCAAGA AGCCACAGAA GCCCATCCCC
4561 CGACCCTTGA ACAAATCCA AGGGATTGTC TTTGATTTCG TCACTCAACA AGCCTTTGAC
15 4621 ATTGTGATCA TGATGCTCAT CTGCCTTAAC ATGGTGACAA TGATGGTGGG GACAGACACT
4681 CAGAGCAAGC AGATGGAGAA CATTCTTTAC TGGATTAATC TGGTCTTTGT CATCTTCTTC
4741 ACCTGCGAGT GTGTGCTCAA AATGTTTGCC TTGAGACACT ACTATTTTAC CATTGGCTGG
4801 AACATCTTTG ACTTTGTGGT GGTCATCCTC TCCATTGTGG GAATGTTCCCT GGCTGATATC
4861 ATTGAGAAGT ACTTCGTCTC CCCAACCCTA TTCCGAGTTA TCCGATTGGC CCGTATTGGG
20 4921 CGCATCTTGC GTCTGATCAA GGGCGCCAAA GGGATCCGCA CCCTGCTCTT TGCCTTAATG
4981 ATGTCGCTGC CCGCCCTGTT CAACATCGGC CTCCTGCTCT TCCTCGTCAT GTTCATCTTC
5041 TCCATTTTTG GCATGTCCAA CTTGCGATAC GTGAAGCACG AGGCCGGCAT TGACGACATG
5101 TTCAACTTCG AGACATTTGG CAACAGCATG ATCTGTTTGT TCCAGATCAC AACGTCTGCT
5161 GGCTGGGATG GCCTGCTGCT GCCAATCCTG AACCGCCCCC CTGACTGCAG CTTGGACAAA
25 5221 GAGCACCAG GGAGTGGCTT CAAAGGGGAC TGTGGGAACC CCTCGGTGGG CATCTTCTTC
5281 TTTGTGAGCT ACATCATCAT CTCCTTCCTG ATTGTGGTGA ACATGTACAT CGCCATCATC
5341 CTGGAGAACT TCAGCGTGGC CACCGAGGAG AGCGCCGACC CTCTGAGTGA GGATGACTTC
5401 GAGACTTTCT ATGAGATCTG GGAGAAGTTT GACCCAGACG CCACCCAGTT CATCGAGTAC
5461 TGTAAGCTGG CAGACTTTGC CGACGCCCTG GAGCACCOCG TCCGAGTACC CAAGCCCAAC
30 5521 ACCATCGAGC TCATCGCCAT GGACCTGCCC ATGGTGAGCG GAGATCGCAT CCACTGCTTG
5581 GACATCCTTT TCGCCTTCAC CAAGCGAGTC CTGGGAGACA GTGGGGAGTT GGACATCCTG
5641 CGGCAGCAGA TGGAGGAGCG GTTCGTGGCA TCCAATCCTT CCAAAGTGTC TTACGAGCCT
5701 ATCACAACCA CTCTGCGGCG CAAGCAGGAG GAGGTGTCTG CAGTGGTCCT GCAGCGTGCC
5761 TACAGGGGAC ACTTGGCTAG GCGGGGCTTC ATCTGCAGAA AGATGGCCTC CAACAAGCTG
35 5821 GAGAATGGAG GCACACACAG AGACAAGAAG GAGAGCACC CGTCCACAGC CTCCCTCCCC
5881 TCTTACGACA GCGTCACAAA GCCAGACAAG GAGAAGCAGC AGCGTGCGGA GGAGGGCAGA

5941 AGGGAAAGAG CCAAGAGGCA AAAAGAGGTC AGGGAGTCCA AGTGCTAGAG GAGGGGAAAG
6001 GAAGCTT

(4) INFORMATION FOR SEQ ID NO:3:

- 5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1978 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: _____
 (D) TOPOLOGY: not relevant
- 10 (ii) MOLECULE TYPE: protein
 (iii) HYPOTHETICAL: YES (although, functionally expressed)
 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: rat
 (F) TISSUE TYPE: dorsal root ganglia
 (G) CELL TYPE: peripheral nerve
- 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

	Met	Ala	Ala	Arg	Leu	Leu	Ala	Pro	Pro	Gly	Pro	Asp	Ser	Phe	Lys
					5					10					15
20	Pro	Phe	Thr	Pro	Glu	Ser	Leu	Ala	Asn	Ile	Glu	Arg	Arg	Ile	Ala
					20					25					30
	Glu	Ser	Lys	Leu	Lys	Lys	Pro	Pro	Lys	Ala	Asp	Gly	Ser	His	Arg
					35					40					45
	Glu	Asp	Asp	Glu	Asp	Ser	Lys	Pro	Lys	Pro	Asn	Ser	Asp	Leu	Glu
25					50					55					60
	Ala	Gly	Lys	Ser	Leu	Pro	Phe	Ile	Tyr	Gly	Asp	Ile	Pro	Gln	Gly
					65					70					75
	Leu	Val	Ala	Val	Pro	Leu	Glu	Asp	Phe	Asp	Pro	Tyr	Tyr	Leu	Thr
					80					85					90
30	Gln	Lys	Thr	Phe	Val	Val	Leu	Asn	Arg	Gly	Lys	Thr	Leu	Phe	Arg
					95					100					105
	Phe	Ser	Ala	Thr	Pro	Ala	Leu	Tyr	Ile	Leu	Ser	Pro	Phe	Asn	Leu
					110					115					120
	Ile	Arg	Arg	Ile	Ala	Ile	Lys	Ile	Leu	Ile	His	Ser	Val	Phe	Ser
35					125					130					135

- 37 -

	Met	Ile	Ile	Met	Cys	Thr	Ile	Leu	Thr	Asn	Cys	Val	Phe	Met	Thr
					140					145					150
	Phe	Ser	Asn	Pro	Pro	Glu	Trp	Ser	Lys	Asn	Val	Glu	Tyr	Thr	Phe
					155					160					165
5	Thr	Gly	Ile	Tyr	Thr	Phe	Glu	Ser	Leu	Val	Lys	Ile	Ile	Ala	Arg
					170					175					180
	Gly	Phe	Cys	Ile	Asp	Gly	Phe	Thr	Phe	Leu	Arg	Asp	Pro	Trp	Asn
					185					190					195
	Trp	Leu	Asp	Phe	Ser	Val	Ile	Met	Met	Ala	Tyr	Val	Thr	Glu	Phe
10					200					205					210
	Val	Asp	Leu	Gly	Asn	Val	Ser	Ala	Leu	Arg	Thr	Phe	Arg	Val	Leu
					215					220					225
	Arg	Ala	Leu	Lys	Thr	Ile	Ser	Val	Ile	Pro	Gly	Leu	Lys	Thr	Ile
					230					235					240
15	Val	Gly	Ala	Leu	Ile	Gln	Ser	Val	Lys	Lys	Leu	Ser	Asp	Val	Met
					245					250					255
	Ile	Leu	Thr	Val	Phe	Cys	Leu	Ser	Val	Phe	Ala	Leu	Ile	Gly	Leu
					260					265					270
	Gln	Leu	Phe	Met	Gly	Asn	Leu	Arg	Asn	Lys	Cys	Val	Val	Trp	Pro
20					275					280					285
	Ile	Asn	Phe	Asn	Glu	Ser	Tyr	Leu	Glu	Asn	Gly	Thr	Arg	Gly	Phe
					290					295					300
	Asp	Trp	Glu	Glu	Tyr	Ile	Asn	Asn	Lys	Thr	Asn	Phe	Tyr	Met	Val
					305					310					315
25	Pro	Gly	Met	Leu	Glu	Pro	Leu	Leu	Cys	Gly	Asn	Ser	Ser	Asp	Ala
					320					325					330
	Gly	Gln	Cys	Pro	Glu	Gly	Phe	Gln	Cys	Met	Lys	Ala	Gly	Arg	Asn
					335					340					345
	Pro	Asn	Tyr	Gly	Tyr	Thr	Ser	Phe	Asp	Thr	Phe	Ser	Trp	Ala	Phe
30					350					355					360
	Leu	Ala	Leu	Phe	Arg	Leu	Met	Thr	Gln	Asp	Tyr	Trp	Glu	Asn	Leu
					365					370					375
	Tyr	Gln	Leu	Thr	Leu	Arg	Ala	Ala	Gly	Lys	Thr	Tyr	Met	Ile	Phe
					380					385					390
35	Phe	Val	Leu	Val	Ile	Phe	Val	Gly	Ser	Phe	Tyr	Leu	Val	Asn	Leu
					395					400					405

- 38 -

	Ile	Leu	Ala	Val	Val	Ala	Met	Ala	Tyr	Glu	Glu	Gln	Asn	Gln	Ala
					410					415					420
	Thr	Leu	Glu	Glu	Ala	Glu	Gln	Lys	Glu	Ala	Glu	Phe	Lys	Ala	Met
					425					430					435
5	Leu	Glu	Gln	Leu	Lys	Lys	Gln	Gln	Glu	Glu	Ala	Gln	Ala	Ala	Ala
					440					445					450
	Met	Ala	Thr	Ser	Ala	Gly	Thr	Val	Ser	Glu	Asp	Ala	Ile	Glu	Glu
					455					460					465
	Glu	Gly	Glu	Asp	Gly	Val	Gly	Ser	Pro	Arg	Ser	Ser	Ser	Glu	Leu
10					470					475					480
	Ser	Lys	Leu	Ser	Ser	Lys	Ser	Ala	Lys	Glu	Arg	Arg	Asn	Arg	Arg
					485					490					495
	Lys	Lys	Arg	Lys	Gln	Lys	Glu	Leu	Ser	Glu	Gly	Glu	Glu	Lys	Gly
					500					505					510
15	Asp	Pro	Glu	Lys	Val	Phe	Lys	Ser	Glu	Ser	Glu	Asp	Gly	Met	Arg
					515					520					525
	Arg	Lys	Ala	Phe	Arg	Leu	Pro	Asp	Asn	Arg	Ile	Gly	Arg	Lys	Phe
					530					535					540
	Ser	Ile	Met	Asn	Gln	Ser	Leu	Leu	Ser	Ile	Pro	Gly	Ser	Pro	Phe
20					545					550					555
	Leu	Ser	Arg	His	Asn	Ser	Lys	Ser	Ser	Ile	Phe	Ser	Phe	Arg	Gly
					560					565					570
	Pro	Gly	Arg	Phe	Arg	Asp	Pro	Gly	Ser	Glu	Asn	Glu	Phe	Ala	Asp
					575					580					585
25	Asp	Glu	His	Ser	Thr	Val	Glu	Glu	Ser	Glu	Gly	Arg	Arg	Asp	Ser
					590					595					600
	Leu	Phe	Ile	Pro	Ile	Arg	Ala	Arg	Glu	Arg	Arg	Ser	Ser	Tyr	Ser
					605					610					615
	Gly	Tyr	Ser	Gly	Tyr	Ser	Gln	Cys	Ser	Arg	Ser	Ser	Arg	Ile	Phe
30					620					625					630
	Pro	Ser	Leu	Arg	Arg	Ser	Val	Lys	Arg	Asn	Ser	Thr	Val	Asp	Cys
					635					640					645
	Asn	Gly	Val	Val	Ser	Leu	Ile	Gly	Pro	Gly	Ser	His	Ile	Gly	Arg
					650					655					660
35	Leu	Leu	Pro	Glu	Ala	Thr	Thr	Glu	Val	Glu	Ile	Lys	Lys	Lys	Gly
					665					670					675

- 39 -

	Pro	Gly	Ser	Leu	Leu	Val	Ser	Met	Asp	Gln	Leu	Ala	Ser	Tyr	Gly
					680					685					690
	Arg	Lys	Asp	Arg	Ile	Asn	Ser	Ile	Met	Ser	Val	Val	Thr	Asn	Thr
					695					700					705
5	Leu	Val	Glu	Glu	Leu	Glu	Glu	Ser	Gln	Arg	Lys	Cys	Pro	Pro	Cys
					710					715					720
	Trp	Tyr	Lys	Phe	Ala	Asn	Thr	Phe	Leu	Ile	Trp	Glu	Cys	His	Pro
					725					730					735
	Tyr	Trp	Ile	Lys	Leu	Lys	Glu	Ile	Val	Asn	Leu	Ile	Val	Met	Asp
10					740					745					750
	Pro	Phe	Val	Asp	Leu	Ala	Ile	Thr	Ile	Cys	Ile	Val	Leu	Asn	Thr
					755					760					765
	Leu	Phe	Met	Ala	Met	Glu	His	His	Pro	Met	Thr	Pro	Gln	Phe	Glu
					770					775					780
15	His	Val	Leu	Ala	Val	Gly	Asn	Leu	Val	Phe	Thr	Gly	Ile	Phe	Thr
					785					790					795
	Ala	Glu	Met	Phe	Leu	Lys	Leu	Ile	Ala	Met	Asp	Pro	Tyr	Tyr	Tyr
					800					805					810
	Phe	Gln	Glu	Gly	Trp	Asn	Ile	Phe	Asp	Gly	Phe	Ile	Val	Ser	Leu
20					815					520					825
	Ser	Leu	Met	Glu	Leu	Ser	Leu	Ala	Asp	Val	Glu	Gly	Leu	Ser	Val
					830					835					840
	Leu	Arg	Ser	Phe	Arg	Leu	Leu	Arg	Val	Phe	Lys	Leu	Ala	Lys	Ser
					845					850					855
25	Trp	Pro	Thr	Leu	Asn	Met	Leu	Ile	Lys	Ile	Ile	Gly	Asn	Ser	Val
					860					865					870
	Gly	Ala	Leu	Gly	Asn	Leu	Thr	Leu	Val	Leu	Ala	Ile	Ile	Val	Phe
					875					880					885
	Ile	Phe	Ala	Val	Val	Gly	Met	Gln	Leu	Phe	Gly	Lys	Ser	Tyr	Lys
30					890					895					900
	Glu	Cys	Val	Cys	Lys	Ile	Asn	Gln	Glu	Cys	Lys	Leu	Pro	Arg	Trp
					905					910					915
	His	Met	Asn	Asp	Phe	Phe	His	Ser	Phe	Leu	Ile	Val	Phe	Arg	Val
					920					925					930
35	Leu	Cys	Gly	Glu	Trp	Ile	Glu	Thr	Met	Trp	Asp	Cys	Met	Glu	Val
					935					940					945

- 40 -

	Ala	Gly	Gln	Ala	Met	Cys	Leu	Ile	Val	Phe	Met	Met	Val	Met	Val
					950					955					960
	Ile	Gly	Asn	Leu	Val	Val	Leu	Asn	Leu	Phe	Leu	Ala	Leu	Leu	Leu
					965					970					975
5	Ser	Ser	Phe	Ser	Ala	Asp	Asn	Leu	Ala	Ala	Thr	Asp	Asp	Asp	Gly
					980					985					990
	Glu	Met	Asn	Asn	Leu	Gln	Ile	Ser	Val	Ile	Arg	Ile	Lys	Lys	Gly
					995					1000					1005
	Val	Ala	Trp	Thr	Lys	Val	Lys	Val	His	Ala	Phe	Met	Gln	Ala	His
10					1010					1015					1020
	Phe	Lys	Gln	Arg	Glu	Ala	Asp	Glu	Val	Lys	Pro	Leu	Asp	Glu	Leu
					1025					1030					1035
	Tyr	Glu	Lys	Lys	Ala	Asn	Cys	Ile	Ala	Asn	His	Thr	Gly	Val	Asp
					1040					1045					1050
15	Ile	His	Arg	Asn	Gly	Asp	Phe	Gln	Lys	Asn	Gly	Asn	Gly	Thr	Thr
					1055					1060					1065
	Ser	Gly	Ile	Gly	Ser	Ser	Val	Glu	Lys	Tyr	Ile	Ile	Asp	Glu	Asp
					1070					1075					1080
	His	Met	Ser	Phe	Ile	Asn	Asn	Pro	Asn	Leu	Thr	Val	Arg	Val	Pro
20					1085					1090					1095
	Ile	Ala	Val	Gly	Glu	Ser	Asp	Phe	Glu	Asn	Leu	Asn	Thr	Glu	Asp
					1100					1105					1110
	Val	Ser	Ser	Glu	Ser	Asp	Pro	Glu	Gly	Ser	Lys	Asp	Lys	Leu	Asp
					1115					1120					1125
25	Asp	Thr	Ser	Ser	Ser	Glu	Gly	Ser	Thr	Ile	Asp	Ile	Lys	Pro	Glu
					1130					1135					1140
	Val	Glu	Glu	Val	Pro	Val	Glu	Gln	Pro	Glu	Glu	Tyr	Leu	Asp	Pro
					1145					1150					1155
	Asp	Ala	Cys	Phe	Thr	Glu	Gly	Cys	Val	Gln	Arg	Phe	Lys	Cys	Cys
30					1160					1165					1170
	Gln	Val	Asn	Ile	Glu	Glu	Gly	Leu	Gly	Lys	Ser	Trp	Trp	Ile	Leu
					1175					1180					1185
	Arg	Lys	Thr	Cys	Phe	Leu	Ile	Val	Glu	His	Asn	Trp	Phe	Glu	Thr
					1190					1195					1200
35	Phe	Ile	Ile	Phe	Met	Ile	Leu	Leu	Ser	Ser	Gly	Ala	Leu	Ala	Phe
					1205					1210					1215

- 41 -

	Glu	Asp	Ile	Tyr	Ile	Glu	Gln	Arg	Lys	Thr	Ile	Arg	Thr	Ile	Leu
					1220					1225					1230
	Glu	Tyr	Ala	Asp	Lys	Val	Phe	Thr	Tyr	Ile	Phe	Ile	Leu	Glu	Met
					1235					1240					1245
5	Leu	Leu	Lys	Trp	Thr	Ala	Tyr	Gly	Phe	Val	Lys	Phe	Phe	Thr	Asn
					1250					1255					1260
	Ala	Trp	Cys	Trp	Leu	Asp	Phe	Leu	Ile	Val	Ala	Val	Ser	Leu	Val
					1265					1270					1275
	Ser	Leu	Ile	Ala	Asn	Ala	Leu	Gly	Tyr	Ser	Glu	Leu	Gly	Ala	Ile
10					1280					1285					1290
	Lys	Ser	Leu	Arg	Thr	Leu	Arg	Ala	Leu	Arg	Pro	Leu	Arg	Ala	Leu
					1295					1300					1305
	Ser	Arg	Phe	Glu	Gly	Met	Arg	Val	Val	Val	Asn	Ala	Leu	Val	Gly
					1310					1315					1320
15	Ala	Ile	Pro	Ser	Ile	Met	Asn	Val	Leu	Leu	Val	Cys	Leu	Ile	Phe
					1325					1330					1335
	Trp	Leu	Ile	Phe	Ser	Ile	Met	Gly	Val	Asn	Leu	Phe	Ala	Gly	Lys
					1340					1345					1350
	Tyr	His	Tyr	Cys	Phe	Asn	Glu	Thr	Ser	Glu	Ile	Arg	Phe	Glu	Ile
20					1355					1360					1365
	Asp	Ile	Val	Asn	Asn	Lys	Thr	Asp	Cys	Glu	Lys	Leu	Met	Glu	Gly
					1370					1375					1380
	Asn	Ser	Thr	Glu	Ile	Arg	Trp	Lys	Asn	Val	Lys	Ile	Asn	Phe	Asp
					1385					1390					1395
25	Asn	Val	Gly	Ala	Gly	Tyr	Leu	Ala	Leu	Leu	Gln	Val	Ala	Thr	Phe
					1400					1405					1410
	Lys	Gly	Trp	Met	Asp	Ile	Met	Tyr	Ala	Ala	Val	Asp	Ser	Arg	Lys
					1415					1420					1425
	Pro	Asp	Glu	Gln	Pro	Asp	Tyr	Glu	Gly	Asn	Ile	Tyr	Met	Tyr	Ile
30					1430					1435					1440
	Tyr	Phe	Val	Ile	Phe	Ile	Ile	Phe	Gly	Ser	Phe	Phe	Thr	Leu	Asn
					1445					1450					1455
	Leu	Phe	Ile	Gly	Val	Ile	Ile	Asp	Asn	Phe	Asn	Gln	Gln	Lys	Lys
					1460					1465					1470
35	Lys	Phe	Gly	Gly	Gln	Asp	Ile	Phe	Met	Thr	Glu	Glu	Gln	Lys	Lys
					1475					1480					1485

- 42 -

	Tyr	Tyr	Asn	Ala	Met	Lys	Lys	Leu	Gly	Ser	Lys	Lys	Pro	Gln	Lys
					1490					1495					1500
	Pro	Ile	Pro	Arg	Pro	Leu	Asn	Lys	Ile	Gln	Gly	Ile	Val	Phe	Asp
					1505					1510					1515
5	Phe	Val	Thr	Gln	Gln	Ala	Phe	Asp	Ile	Val	Ile	Met	Met	Leu	Ile
					1520					1525					1530
	Cys	Leu	Asn	Met	Val	Thr	Met	Met	Val	Glu	Thr	Asp	Thr	Gln	Ser
					1535					1540					1545
10	Lys	Gln	Met	Glu	Asn	Ile	Leu	Tyr	Trp	Ile	Asn	Leu	Val	Phe	Val
					1550					1555					1560
	Ile	Phe	Phe	Thr	Cys	Glu	Cys	Val	Leu	Lys	Met	Phe	Ala	Leu	Arg
					1565					1570					1575
	His	Tyr	Tyr	Phe	Thr	Ile	Gly	Trp	Asn	Ile	Phe	Asp	Phe	Val	Val
					1580					1585					1590
15	Val	Ile	Leu	Ser	Ile	Val	Gly	Met	Phe	Leu	Ala	Asp	Ile	Ile	Glu
					1595					1600					1605
	Lys	Tyr	Phe	Val	Ser	Pro	Thr	Leu	Phe	Arg	Val	Ile	Arg	Leu	Ala
					1610					1615					1620
20	Arg	Ile	Gly	Arg	Ile	Leu	Arg	Leu	Ile	Lys	Gly	Ala	Lys	Gly	Ile
					1625					1630					1635
	Arg	Thr	Leu	Leu	Phe	Ala	Leu	Met	Met	Ser	Leu	Pro	Ala	Leu	Phe
					1640					1645					1650
	Asn	Ile	Gly	Leu	Leu	Leu	Phe	Leu	Val	Met	Phe	Ile	Phe	Ser	Ile
					1655					1660					1665
25	Phe	Gly	Met	Ser	Asn	Phe	Ala	Tyr	Val	Lys	His	Glu	Ala	Gly	Ile
					1670					1675					1680
	Asp	Asp	Met	Phe	Asn	Phe	Glu	Thr	Phe	Gly	Asn	Ser	Met	Ile	Cys
					1685					1690					1695
30	Leu	Phe	Gln	Ile	Thr	Thr	Ser	Ala	Gly	Trp	Asp	Gly	Leu	Leu	Leu
					1700					1705					1710
	Pro	Ile	Leu	Asn	Arg	Pro	Pro	Asp	Cys	Ser	Leu	Asp	Lys	Glu	His
					1715					1720					1725
	Pro	Gly	Ser	Gly	Phe	Lys	Gly	Asp	Cys	Gly	Asn	Pro	Ser	Val	Gly
					1730					1735					1740
35	Ile	Phe	Phe	Phe	Val	Ser	Tyr	Ile	Ile	Ile	Ser	Phe	Leu	Ile	Val
					1745					1750					1755

- 43 -

	Val	Asn	Met	Tyr	Ile	Ala	Ile	Ile	Leu	Glu	Asn	Phe	Ser	Val	Ala
					1760					1765					1770
	Thr	Glu	Glu	Ser	Ala	Asp	Pro	Leu	Ser	Glu	Asp	Asp	Phe	Glu	Thr
					1775					1780					1785
5	Phe	Tyr	Glu	Ile	Trp	Glu	Lys	Phe	Asp	Pro	Asp	Ala	Thr	Gln	Phe
					1790					1795					1800
	Ile	Glu	Tyr	Cys	Lys	Leu	Ala	Asp	Phe	Ala	Asp	Ala	Leu	Glu	His
					1805					1810					1815
	Pro	Leu	Arg	Val	Pro	Lys	Pro	Asn	Thr	Ile	Glu	Leu	Ile	Ala	Met
10					1820					1825					1830
	Asp	Leu	Pro	Met	Val	Ser	Gly	Asp	Arg	Ile	His	Cys	Leu	Asp	Ile
					1835					1840					1845
	Leu	Phe	Ala	Phe	Thr	Lys	Arg	Val	Leu	Gly	Asp	Ser	Gly	Glu	Leu
					1850					1855					1860
15	Asp	Ile	Leu	Arg	Gln	Gln	Met	Glu	Glu	Arg	Phe	Val	Ala	Ser	Asn
					1865					1870					1875
	Pro	Ser	Lys	Val	Ser	Tyr	Glu	Pro	Ile	Thr	Thr	Thr	Leu	Arg	Arg
					1880					1885					1890
	Lys	Gln	Glu	Glu	Val	Ser	Ala	Val	Val	Leu	Gln	Arg	Ala	Tyr	Arg
20					1895					1900					1905
	Gly	His	Leu	Ala	Arg	Arg	Gly	Phe	Ile	Cys	Arg	Lys	Met	Ala	Ser
					1910					1915					1920
	Asn	Lys	Leu	Glu	Asn	Gly	Gly	Thr	His	Arg	Asp	Lys	Lys	Glu	Ser
					1925					1930					1935
25	Thr	Pro	Ser	Thr	Ala	Ser	Leu	Pro	Ser	Tyr	Asp	Ser	Val	Thr	Lys
					1940					1945					1950
	Pro	Asp	Lys	Glu	Lys	Gln	Gln	Arg	Ala	Glu	Glu	Gly	Arg	Arg	Glu
					1955					1960					1965
	Arg	Ala	Lys	Arg	Gln	Lys	Glu	Val	Arg	Glu	Ser	Lys	Cys		
30					1970					1975					

(5) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH: 1988 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:

- 44 -

- (D) TOPOLOGY: not relevant
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: YES
(vi) ORIGINAL SOURCE:
5 (A) ORGANISM: rat
(F) TISSUE TYPE: dorsal root ganglia
(G) CELL TYPE: peripheral nerve
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

10	Met	Ala	Ala	Arg	Leu	Leu	Ala	Pro	Pro	Gly	Pro	Asp	Ser	Phe	Lys
					5					10					15
	Pro	Phe	Thr	Pro	Glu	Ser	Leu	Ala	Asn	Ile	Glu	Arg	Arg	Ile	Ala
					20					25					30
	Glu	Ser	Lys	Leu	Lys	Lys	Pro	Pro	Lys	Ala	Asp	Gly	Ser	His	Arg
15					35					40					45
	Glu	Asp	Asp	Glu	Asp	Ser	Lys	Pro	Lys	Pro	Asn	Ser	Asp	Leu	Glu
					50					55					60
	Ala	Gly	Lys	Ser	Leu	Pro	Phe	Ile	Tyr	Gly	Asp	Ile	Pro	Gln	Gly
					65					70					75
20	Leu	Val	Ala	Val	Pro	Leu	Glu	Asp	Phe	Asp	Pro	Tyr	Tyr	Leu	Thr
					80					85					90
	Gln	Lys	Thr	Phe	Val	Val	Leu	Asn	Arg	Gly	Lys	Thr	Leu	Phe	Arg
					95					100					105
	Phe	Ser	Ala	Thr	Pro	Ala	Leu	Tyr	Ile	Leu	Ser	Pro	Phe	Asn	Leu
25					110					115					120
	Ile	Arg	Arg	Ile	Ala	Ile	Lys	Ile	Leu	Ile	His	Ser	Val	Phe	Ser
					125					130					135
	Met	Ile	Ile	Met	Cys	Thr	Ile	Leu	Thr	Asn	Cys	Val	Phe	Met	Thr
					140					145					150
30	Phe	Ser	Asn	Pro	Pro	Glu	Trp	Ser	Lys	Asn	Val	Glu	Tyr	Thr	Phe
					155					160					165
	Thr	Gly	Ile	Tyr	Thr	Phe	Glu	Ser	Leu	Val	Lys	Ile	Ile	Ala	Arg
					170					175					180
	Gly	Phe	Cys	Ile	Asp	Gly	Phe	Thr	Phe	Leu	Arg	Asp	Pro	Trp	Asn
35					185					190					195

- 45 -

	Trp	Leu	Asp	Phe	Ser	Val	Ile	Met	Met	Ala	Tyr	Val	Thr	Glu	Phe
					200					205					210
	Val	Asp	Leu	Gly	Asn	Val	Ser	Ala	Leu	Arg	Thr	Phe	Arg	Val	Leu
					215					220					225
5	Arg	Ala	Leu	Lys	Thr	Ile	Ser	Val	Ile	Pro	Gly	Leu	Lys	Thr	Ile
					230					235					240
	Val	Gly	Ala	Leu	Ile	Gln	Ser	Val	Lys	Lys	Leu	Ser	Asp	Val	Met
					245					250					255
	Ile	Leu	Thr	Val	Phe	Cys	Leu	Ser	Val	Phe	Ala	Leu	Ile	Gly	Leu
10					260					265					270
	Gln	Leu	Phe	Met	Gly	Asn	Leu	Arg	Asn	Lys	Cys	Val	Val	Trp	Pro
					275					280					285
	Ile	Asn	Phe	Asn	Glu	Ser	Tyr	Leu	Glu	Asn	Gly	Thr	Arg	Gly	Phe
					290					295					300
15	Asp	Trp	Glu	Glu	Tyr	Ile	Asn	Asn	Lys	Thr	Asn	Phe	Tyr	Met	Val
					305					310					315
	Pro	Gly	Met	Leu	Glu	Pro	Leu	Leu	Cys	Gly	Asn	Ser	Ser	Asp	Ala
					320					325					330
	Gly	Gln	Cys	Pro	Glu	Gly	Phe	Gln	Cys	Met	Lys	Ala	Gly	Arg	Asn
20					335					340					345
	Pro	Asn	Tyr	Gly	Tyr	Thr	Ser	Phe	Asp	Thr	Phe	Ser	Trp	Ala	Phe
					350					355					360
	Leu	Ala	Leu	Phe	Arg	Leu	Met	Thr	Gln	Asp	Tyr	Trp	Glu	Asn	Leu
					365					370					375
25	Tyr	Gln	Leu	Thr	Leu	Arg	Ala	Ala	Gly	Lys	Thr	Tyr	Met	Ile	Phe
					380					385					390
	Phe	Val	Leu	Val	Ile	Phe	Val	Gly	Ser	Phe	Tyr	Leu	Val	Asn	Leu
					395					400					405
	Ile	Leu	Ala	Val	Val	Ala	Met	Ala	Tyr	Glu	Glu	Gln	Asn	Gln	Ala
30					410					415					420
	Thr	Leu	Glu	Glu	Ala	Glu	Gln	Lys	Glu	Ala	Glu	Phe	Lys	Ala	Met
					425					430					435
	Leu	Glu	Gln	Leu	Lys	Lys	Gln	Gln	Glu	Glu	Ala	Gln	Ala	Ala	Ala
					440					445					450
35	Met	Ala	Thr	Ser	Ala	Gly	Thr	Val	Ser	Glu	Asp	Ala	Ile	Glu	Glu
					455					460					465

	Glu	Gly	Glu	Asp	Gly	Val	Gly	Ser	Pro	Arg	Ser	Ser	Ser	Glu	Leu
					470					475					480
	Ser	Lys	Leu	Ser	Ser	Lys	Ser	Ala	Lys	Glu	Arg	Arg	Asn	Arg	Arg
					485					490					495
5	Lys	Lys	Arg	Lys	Gln	Lys	Glu	Leu	Ser	Glu	Gly	Glu	Glu	Lys	Gly
					500					505					510
	Asp	Pro	Glu	Lys	Val	Phe	Lys	Ser	Glu	Ser	Glu	Asp	Gly	Met	Arg
					515					520					525
10	Arg	Lys	Ala	Phe	Arg	Leu	Pro	Asp	Asn	Arg	Ile	Gly	Arg	Lys	Phe
					530					535					540
	Ser	Ile	Met	Asn	Gln	Ser	Leu	Leu	Ser	Ile	Pro	Gly	Ser	Pro	Phe
					545					550					555
	Leu	Ser	Arg	His	Asn	Ser	Lys	Ser	Ser	Ile	Phe	Ser	Phe	Arg	Gly
					560					565					570
15	Pro	Gly	Arg	Phe	Arg	Asp	Pro	Gly	Ser	Glu	Asn	Glu	Phe	Ala	Asp
					575					580					585
	Asp	Glu	His	Ser	Thr	Val	Glu	Glu	Ser	Glu	Gly	Arg	Arg	Asp	Ser
					590					595					600
20	Leu	Phe	Ile	Pro	Ile	Arg	Ala	Arg	Glu	Arg	Arg	Ser	Ser	Tyr	Ser
					605					610					615
	Gly	Tyr	Ser	Gly	Tyr	Ser	Gln	Cys	Ser	Arg	Ser	Ser	Arg	Ile	Phe
					620					625					630
	Pro	Ser	Leu	Arg	Arg	Ser	Val	Lys	Arg	Asn	Ser	Thr	Val	Asp	Cys
					635					640					645
25	Asn	Gly	Val	Val	Ser	Leu	Ile	Gly	Pro	Gly	Ser	His	Ile	Gly	Arg
					650					655					660
	Leu	Leu	Pro	Glu	Val	Lys	Ile	Asp	Lys	Ala	Ala	Thr	Asp	Ser	Ala
					665					670					675
30	Thr	Thr	Glu	Val	Glu	Ile	Lys	Lys	Lys	Gly	Pro	Gly	Ser	Leu	Leu
					680					685					690
	Val	Ser	Met	Asp	Gln	Leu	Ala	Ser	Tyr	Gly	Arg	Lys	Asp	Arg	Ile
					695					700					705
	Asn	Ser	Ile	Met	Ser	Val	Val	Thr	Asn	Thr	Leu	Val	Glu	Glu	Leu
					710					715					720
35	Glu	Glu	Ser	Gln	Arg	Lys	Cys	Pro	Pro	Cys	Trp	Tyr	Lys	Phe	Ala
					725					730					735

	Asn	Thr	Phe	Leu	Ile	Trp	Glu	Cys	His	Pro	Tyr	Trp	Ile	Lys	Leu
					740					745					750
	Lys	Glu	Ile	Val	Asn	Leu	Ile	Val	Met	Asp	Pro	Phe	Val	Asp	Leu
					755					760					765
5	Ala	Ile	Thr	Ile	Cys	Ile	Val	Leu	Asn	Thr	Leu	Phe	Met	Ala	Met
					770					775					780
	Glu	His	His	Pro	Met	Thr	Pro	Gln	Phe	Glu	His	Val	Leu	Ala	Val
					785					790					795
	Gly	Asn	Leu	Val	Phe	Thr	Gly	Ile	Phe	Thr	Ala	Glu	Met	Phe	Leu
10					800					805					810
	Lys	Leu	Ile	Ala	Met	Asp	Pro	Tyr	Tyr	Tyr	Phe	Gln	Glu	Gly	Trp
					815					820					825
	Asn	Ile	Phe	Asp	Gly	Phe	Ile	Val	Ser	Leu	Ser	Leu	Met	Glu	Leu
					830					835					840
15	Ser	Leu	Ala	Asp	Val	Glu	Gly	Leu	Ser	Val	Leu	Arg	Ser	Phe	Arg
					845					850					855
	Leu	Leu	Arg	Val	Phe	Lys	Leu	Ala	Lys	Ser	Trp	Pro	Thr	Leu	Asn
					860					865					870
	Met	Leu	Ile	Lys	Ile	Ile	Gly	Asn	Ser	Val	Gly	Ala	Leu	Gly	Asn
20					875					880					885
	Leu	Thr	Leu	Val	Leu	Ala	Ile	Ile	Val	Phe	Ile	Phe	Ala	Val	Val
					890					895					900
	Gly	Met	Gln	Leu	Phe	Gly	Lys	Ser	Tyr	Lys	Glu	Cys	Val	Cys	Lys
					905					910					915
25	Ile	Asn	Gln	Glu	Cys	Lys	Leu	Pro	Arg	Trp	His	Met	Asn	Asp	Phe
					920					925					930
	Phe	His	Ser	Phe	Leu	Ile	Val	Phe	Arg	Val	Leu	Cys	Gly	Glu	Trp
					935					940					945
	Ile	Glu	Thr	Met	Trp	Asp	Cys	Met	Glu	Val	Ala	Gly	Gln	Ala	Met
30					950					955					960
	Cys	Leu	Ile	Val	Phe	Met	Met	Val	Met	Val	Ile	Gly	Asn	Leu	Val
					965					970					975
	Val	Leu	Asn	Leu	Phe	Leu	Ala	Leu	Leu	Leu	Ser	Ser	Phe	Ser	Ala
					980					985					990
35	Asp	Asn	Leu	Ala	Ala	Thr	Asp	Asp	Asp	Gly	Glu	Met	Asn	Asn	Leu
					995					1000					1005

	Gln	Ile	Ser	Val	Ile	Arg	Ile	Lys	Lys	Gly	Val	Ala	Trp	Thr	Lys
					1010					1015					1020
	Val	Lys	Val	His	Ala	Phe	Met	Gln	Ala	His	Phe	Lys	Gln	Arg	Glu
					1025					1030					1035
5	Ala	Asp	Glu	Val	Lys	Pro	Leu	Asp	Glu	Leu	Tyr	Glu	Lys	Lys	Ala
					1040					1045					1050
	Asn	Cys	Ile	Ala	Asn	His	Thr	Gly	Val	Asp	Ile	His	Arg	Asn	Gly
					1055					1060					1065
	Asp	Phe	Gln	Lys	Asn	Gly	Asn	Gly	Thr	Thr	Ser	Gly	Ile	Gly	Ser
10					1070					1075					1080
	Ser	Val	Glu	Lys	Tyr	Ile	Ile	Asp	Glu	Asp	His	Met	Ser	Phe	Ile
					1085					1090					1095
	Asn	Asn	Pro	Asn	Leu	Thr	Val	Arg	Val	Pro	Ile	Ala	Val	Gly	Glu
					1100					1105					1110
15	Ser	Asp	Phe	Glu	Asn	Leu	Asn	Thr	Glu	Asp	Val	Ser	Ser	Glu	Ser
					1115					1120					1125
	Asp	Pro	Glu	Gly	Ser	Lys	Asp	Lys	Leu	Asp	Asp	Thr	Ser	Ser	Ser
					1130					1135					1140
	Glu	Gly	Ser	Thr	Ile	Asp	Ile	Lys	Pro	Glu	Val	Glu	Glu	Val	Pro
20					1145					1150					1155
	Val	Glu	Gln	Pro	Glu	Glu	Tyr	Leu	Asp	Pro	Asp	Ala	Cys	Phe	Thr
					1160					1165					1170
	Glu	Gly	Cys	Val	Gln	Arg	Phe	Lys	Cys	Cys	Gln	Val	Asn	Ile	Glu
					1175					1180					1185
25	Glu	Gly	Leu	Gly	Lys	Ser	Trp	Trp	Ile	Leu	Arg	Lys	Thr	Cys	Phe
					1190					1195					1200
	Leu	Ile	Val	Glu	His	Asn	Trp	Phe	Glu	Thr	Phe	Ile	Ile	Phe	Met
					1205					1210					1215
	Ile	Leu	Leu	Ser	Ser	Gly	Ala	Leu	Ala	Phe	Glu	Asp	Ile	Tyr	Ile
30					1220					1225					1230
	Glu	Gln	Arg	Lys	Thr	Ile	Arg	Thr	Ile	Leu	Glu	Tyr	Ala	Asp	Lys
					1235					1240					1245
	Val	Phe	Thr	Tyr	Ile	Phe	Ile	Leu	Glu	Met	Leu	Leu	Lys	Trp	Thr
					1250					1255					1260
35	Ala	Tyr	Gly	Phe	Val	Lys	Phe	Phe	Thr	Asn	Ala	Trp	Cys	Trp	Leu
					1265					1270					1275

	Asp	Phe	Leu	Ile	Val	Ala	Val	Ser	Leu	Val	Ser	Leu	Ile	Ala	Asn
					1280					1285					1290
	Ala	Leu	Gly	Tyr	Ser	Glu	Leu	Gly	Ala	Ile	Lys	Ser	Leu	Arg	Thr
					1295					1300					1305
5	Leu	Arg	Ala	Leu	Arg	Pro	Leu	Arg	Ala	Leu	Ser	Arg	Phe	Glu	Gly
					1310					1315					1320
	Met	Arg	Val	Val	Val	Asn	Ala	Leu	Val	Gly	Ala	Ile	Pro	Ser	Ile
					1325					1330					1335
	Met	Asn	Val	Leu	Leu	Val	Cys	Leu	Ile	Phe	Trp	Leu	Ile	Phe	Ser
10					1340					1345					1350
	Ile	Met	Gly	Val	Asn	Leu	Phe	Ala	Gly	Lys	Tyr	His	Tyr	Cys	Phe
					1355					1360					1365
	Asn	Glu	Thr	Ser	Glu	Ile	Arg	Phe	Glu	Ile	Asp	Ile	Val	Asn	Asn
					1370					1375					1380
15	Lys	Thr	Asp	Cys	Glu	Lys	Leu	Met	Glu	Gly	Asn	Ser	Thr	Glu	Ile
					1385					1390					1395
	Arg	Trp	Lys	Asn	Val	Lys	Ile	Asn	Phe	Asp	Asn	Val	Gly	Ala	Gly
					1400					1405					1410
	Tyr	Leu	Ala	Leu	Leu	Gln	Val	Ala	Thr	Phe	Lys	Gly	Trp	Met	Asp
20					1415					1420					1425
	Ile	Met	Tyr	Ala	Ala	Val	Asp	Ser	Arg	Lys	Pro	Asp	Glu	Gln	Pro
					1430					1435					1440
	Asp	Tyr	Glu	Gly	Asn	Ile	Tyr	Met	Tyr	Ile	Tyr	Phe	Val	Ile	Phe
					1445					1450					1455
25	Ile	Ile	Phe	Gly	Ser	Phe	Phe	Thr	Leu	Asn	Leu	Phe	Ile	Gly	Val
					1460					1465					1470
	Ile	Ile	Asp	Asn	Phe	Asn	Gln	Gln	Lys	Lys	Lys	Phe	Gly	Gly	Gln
					1475					1480					1485
	Asp	Ile	Phe	Met	Thr	Glu	Glu	Gln	Lys	Lys	Tyr	Tyr	Asn	Ala	Met
30					1490					1495					1500
	Lys	Lys	Leu	Gly	Ser	Lys	Lys	Pro	Gln	Lys	Pro	Ile	Pro	Arg	Pro
					1505					1510					1515
	Leu	Asn	Lys	Ile	Gln	Gly	Ile	Val	Phe	Asp	Phe	Val	Thr	Gln	Gln
					1520					1525					1530
35	Ala	Phe	Asp	Ile	Val	Ile	Met	Met	Leu	Ile	Cys	Leu	Asn	Met	Val
					1535					1540					1545

- 50 -

	Thr	Met	Met	Val	Glu	Thr	Asp	Thr	Gln	Ser	Lys	Gln	Met	Glu	Asn
					1550					1555					1560
	Ile	Leu	Tyr	Trp	Ile	Asn	Leu	Val	Phe	Val	Ile	Phe	Phe	Thr	Cys
					1565					1570					1575
5	Glu	Cys	Val	Leu	Lys	Met	Phe	Ala	Leu	Arg	His	Tyr	Tyr	Phe	Thr
					1580					1585					1590
	Ile	Gly	Trp	Asn	Ile	Phe	Asp	Phe	Val	Val	Val	Ile	Leu	Ser	Ile
					1595					1600					1605
10	Val	Gly	Met	Phe	Leu	Ala	Asp	Ile	Ile	Glu	Lys	Tyr	Phe	Val	Ser
					1610					1615					1620
	Pro	Thr	Leu	Phe	Arg	Val	Ile	Arg	Leu	Ala	Arg	Ile	Gly	Arg	Ile
					1625					1630					1635
	Leu	Arg	Leu	Ile	Lys	Gly	Ala	Lys	Gly	Ile	Arg	Thr	Leu	Leu	Phe
					1640					1645					1650
15	Ala	Leu	Met	Met	Ser	Leu	Pro	Ala	Leu	Phe	Asn	Ile	Gly	Leu	Leu
					1655					1660					1665
	Leu	Phe	Leu	Val	Met	Phe	Ile	Phe	Ser	Ile	Phe	Gly	Met	Ser	Asn
					1670					1675					1680
20	Phe	Ala	Tyr	Val	Lys	His	Glu	Ala	Gly	Ile	Asp	Asp	Met	Phe	Asn
					1685					1690					1695
	Phe	Glu	Thr	Phe	Gly	Asn	Ser	Met	Ile	Cys	Leu	Phe	Gln	Ile	Thr
					1700					1705					1710
	Thr	Ser	Ala	Gly	Trp	Asp	Gly	Leu	Leu	Leu	Pro	Ile	Leu	Asn	Arg
					1715					1720					1725
25	Pro	Pro	Asp	Cys	Ser	Leu	Asp	Lys	Glu	His	Pro	Gly	Ser	Gly	Phe
					1730					1735					1740
	Lys	Gly	Asp	Cys	Gly	Asn	Pro	Ser	Val	Gly	Ile	Phe	Phe	Phe	Val
					1745					1750					1755
30	Ser	Tyr	Ile	Ile	Ile	Ser	Phe	Leu	Ile	Val	Val	Asn	Met	Tyr	Ile
					1760					1765					1770
	Ala	Ile	Ile	Leu	Glu	Asn	Phe	Ser	Val	Ala	Thr	Glu	Glu	Ser	Ala
					1775					1780					1785
	Asp	Pro	Leu	Ser	Glu	Asp	Asp	Phe	Glu	Thr	Phe	Tyr	Glu	Ile	Trp
					1790					1795					1800
35	Glu	Lys	Phe	Asp	Pro	Asp	Ala	Thr	Gln	Phe	Ile	Glu	Tyr	Cys	Lys
					1805					1810					1815

- 51 -

	Leu	Ala	Asp	Phe	Ala	Asp	Ala	Leu	Glu	His	Pro	Leu	Arg	Val	Pro
					1820					1825					1830
	Lys	Pro	Asn	Thr	Ile	Glu	Leu	Ile	Ala	Met	Asp	Leu	Pro	Met	Val
					1935					1840					1845
5	Ser	Gly	Asp	Arg	Ile	His	Cys	Leu	Asp	Ile	Leu	Phe	Ala	Phe	Thr
					1850					1855					1860
	Lys	Arg	Val	Leu	Gly	Asp	Ser	Gly	Glu	Leu	Asp	Ile	Leu	Arg	Gln
					1865					1870					1875
	Gln	Met	Glu	Glu	Arg	Phe	Val	Ala	Ser	Asn	Pro	Ser	Lys	Val	Ser
10					1880					1885					1890
	Tyr	Glu	Pro	Ile	Thr	Thr	Thr	Leu	Arg	Arg	Lys	Gln	Glu	Glu	Val
					1895					1900					1905
	Ser	Ala	Val	Val	Leu	Gln	Arg	Ala	Tyr	Arg	Gly	His	Leu	Ala	Arg
					1910					1915					1920
15	Arg	Gly	Phe	Ile	Cys	Arg	Lys	Met	Ala	Ser	Asn	Lys	Leu	Glu	Asn
					1925					1930					1935
	Gly	Gly	Thr	His	Arg	Asp	Lys	Lys	Glu	Ser	Thr	Pro	Ser	Thr	Ala
					1940					1945					1950
	Ser	Leu	Pro	Ser	Tyr	Asp	Ser	Val	Thr	Lys	Pro	Asp	Lys	Glu	Lys
20					1955					1960					1965
	Gln	Gln	Arg	Ala	Glu	Glu	Gly	Arg	Arg	Glu	Arg	Ala	Lys	Arg	Gln
					1970					1975					1980
	Lys	Glu	Val	Arg	Glu	Ser	Lys	Cys							
					1985										

25

(6) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 696 base pairs

(B) TYPE: nucleic acid

30

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RT-PCR

(A) DESCRIPTION: /desc = „DNA probe“

(iii) HYPOTHETICAL: NO

35

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: rat

(F) TISSUE TYPE: dorsal root ganglia

(G) CELL TYPE: peripheral nerve

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

1 CTCAACATGG TTACTATGAT GGTGGAGACA GAACTCAGA GCAAGCAGAT
51 GGAGAACATT CTTTACTGGA TTAATCTGGT CTTTGTATC TTCTTCACCT
101 GCGAGTGTGT GCTCAAAATG TTTGCCTTGA GAACTACTA TTTCACCATT
151 GGCTGGAACA TCTTTGACTT TGTGGTGGTC ATCCTCTCCA TTGTGGGAAT
201 GTTCCTGGCT GATATCATTG AGAAGTACTT CGTCTCCCCA ACCCTATTCC
251 GAGTTATCCG ATTGGCCCCG ATTGGGCGCA TCTTGCCTCT GATCAAGGGG
301 GCCAAAGGGA TCCGCACCCT GCTCTTTGGC CTTAATGATG TCGCTGGCCG
351 CCCTGTTCAA CATCGCCTCC TGCTCTTCCT CGTCATGTTC ATCTTCTCCA
401 TTTTGGCAT GTCCAATTG GCATACGTGA AGCAGAGGC CGGCATTGAC
451 GACATGTTCA ACTTCGAGAC ATTTGGCAAC AGCATGATCT GTTTGTTCCA
501 GATCACAACG TCTGCTGGCT GGGATGGCCT GCTGCTGCCA ATCCTGAACC
551 GCCCCCTGA CTGCAGCTTG GACAAAGAGC ACCCAGGGAG TGGCTTCAAA
601 GGGGACTGTG GGAACCCCTC GGTGGGCATC TTCTTCTTTG TGAGCTACAT
651 CATCATCTCC TTCCTGATTG TGGTGAACAT GTACATCGCA GTCATC

(7) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 232 amino acids

(B) TYPE: amino acid

5 (C) STRANDEDNESS:

(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(vi) ORIGINAL SOURCE:

10 (A) ORGANISM: rat

(F) TISSUE TYPE: dorsal root ganglia

(G) CELL TYPE: peripheral nerve

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

15	Leu	Asn	Met	Val	Thr	Met	Met	Val	Glu	Thr	Asp	Thr	Gln	Ser	Lys
					5					10					15
	Gln	Met	Glu	Asn	Ile	Leu	Tyr	Trp	Ile	Asn	Leu	Val	Phe	Val	Ile
					20					25					30
	Phe	Phe	Thr	Cys	Glu	Cys	Val	Leu	Lys	Met	Phe	Ala	Leu	Arg	His
20					35					40					45
	Tyr	Tyr	Phe	Thr	Ile	Gly	Trp	Asn	Ile	Phe	Asp	Phe	Val	Val	Val
					50					55					60
	Ile	Leu	Ser	Ile	Val	Gly	Met	Phe	Leu	Ala	Asp	Ile	Ile	Glu	Lys
					65					70					75
25	Tyr	Phe	Val	Ser	Pro	Thr	Leu	Phe	Arg	Val	Ile	Arg	Leu	Ala	Arg
					80					85					90
	Ile	Gly	Arg	Ile	Leu	Arg	Leu	Ile	Lys	Gly	Ala	Lys	Gly	Ile	Arg
					95					100					105
	Thr	Leu	Leu	Phe	Gly	Leu	Asn	Asp	Val	Ala	Gly	Arg	Pro	Val	Gln
30					110					115					120
	His	Arg	Leu	Leu	Leu	Phe	Leu	Val	Met	Phe	Ile	Phe	Ser	Ile	Phe
					125					130					135
	Gly	Met	Ser	Asn	Phe	Ala	Tyr	Val	Lys	His	Glu	Ala	Gly	Ile	Asp
					140					145					150
35	Asp	Met	Phe	Asn	Phe	Glu	Thr	Phe	Gly	Asn	Ser	Met	Ile	Cys	Leu
					155					160					165

	Phe	Gln	Ile	Thr	Thr	Ser	Ala	Gly	Trp	Asp	Gly	Leu	Leu	Leu	Pro
					170					175					180
	Ile	Leu	Asn	Arg	Pro	Pro	Asp	Cys	Ser	Leu	Asp	Lys	Glu	His	Pro
					185					190					195
5	Gly	Ser	Gly	Phe	Lys	Gly	Asp	Cys	Gly	Asn	Pro	Ser	Val	Gly	Ile
					200					205					210
	Phe	Phe	Phe	Val	Ser	Tyr	Ile	Ile	Ile	Ser	Phe	Leu	Ile	Val	Val
					215					220					225
	Asn	Met	Tyr	Ile	Ala	Val	Ile								
10					230										

(8) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- 15 (A) LENGTH: 6556 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- 20 (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: rat
- (F) TISSUE TYPE: Dorsal root ganglia
- (G) CELL TYPE: Peripheral nerve
- 25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

	CCAAGATGGC	GCCCACCGCA	GTCCCGCCCG	CCGCAGCCTC	GGCGCCTCTG	50
	CAGTCCGGCC	GCGCCTCCCG	GGCCCCGCGC	TAGGGCCGCT	GCCGCCTCGC	100
	CCGCCGCCGC	CGCCGCCAGC	TGACCTGTCC	CGGACACATA	ACTAACGAAG	150
30	CTGCTGCAGG	ATGAGAAGAT	GGCAGCGCGG	CTGCTCGCAC	CACCAGGCCC	200
	TGATAGTTTC	AAGCCTTTCA	CCCCTGAGTC	GCTGGCAAAC	ATCGAGAGGC	250
	GTATTGCCGA	GAGCAAGCTC	AAGAAACCAC	CAAAGGCGGA	TGGCAGCCAC	300
	CGGGAGGACG	ATGAAGACAG	CAAGCCCAAG	CCAAACAGTG	ACCTGGAGGC	350
	TGGGAAGAGT	TTGCCTTTCA	TCTACGGGGA	CATCCCGCAA	GGCCTGGTTG	400
35	CGGTTCCCCT	GGAGGACTTT	GACCCTTACT	ATTTGACGCA	GAAAACCTTT	450
	GTAGTATTAA	ACAGAGGGAA	AACTCTCTTC	AGATTTAGTG	CCACACCTGC	500

	CTTGTACATT	TTAAGCCCTT	TTAACCTGAT	AAGAAGAATA	GCTATTAAAA	550
	TTTTGATACA	CTCAGTTTTC	AGCATGATCA	TCATGTGCAC	CATCCTGACC	600
	AACTGTGTGT	TCATGACCTT	TAGTAACCCT	CCAGAATGGT	CCAAGAATGT	650
	GGAGTACACA	TTCACAGGGA	TTTACACATT	TGAATCACTA	GTGAAAATCA	700
5	TCGCAAGAGG	TTTCTGCATA	GACGGCTTCA	CCTTCTTGCG	AGACCCGTGG	750
	AACTGGTTAG	ACTTCAGTGT	CATCATGATG	GCATATGTGA	CAGAGTTTGT	800
	GGACCTGGGC	AATGTCTCAG	CGCTGAGAAC	ATTCAGGGTT	CTCCGAGCTT	850
	TGAAAACAT	CTCTGTAATT	CCAGGCCTGA	AGACAATCGT	GGGCGCCCTA	900
	ATCCAGTCCG	TGAAGAAGCT	GTCGGACGTG	ATGATCCTGA	CAGTGTCTCTG	950
10	CCTGAGTGTT	TTCGCCCTGA	TTGGCCTGCA	GCTCTTCATG	GGGAACCTT-	999
	CGAAACAAGT	GTGTCGTGTG	GCCCATAAAC	TTCAACGAGA	GCTACCTGGA	1049
	GAACGGCACC	AGAGGCTTTG	ACTGGGAGGA	ATATATCAAC	AATAAAACAA	1099
	ACTTTTACAT	GGTTCCTGGC	ATGCTAGAAC	CCTTGCTCTG	CGGGAACAGT	1149
	TCTGATGCTG	GGCAATGCCC	AGAGGGATTC	CAGTGCATGA	AAGCAGGAAG	1199
15	GAACCCCAAC	TACGGTTACA	CCAGCTTTGA	CACCTTCAGC	TGGGCCCTTCT	1249
	TGGCATTATT	CCGCCTTATG	ACCCAGGACT	ATTGGGAGAA	CTTATACCAG	1299
	CTGACCTTAC	GAGCCGCTGG	GAAAACGTAC	ATGATCTTCT	TTGTCTTGGT	1349
	CATCTTCGTG	GGTTCTTTCT	ATCTGGTGAA	CTTGATCTTG	GCTGTGGTGG	1399
	CCATGGCTTA	TGAGGAACAG	AACCAGGCAA	CACTGGAGGA	GGCAGAGCAA	1449
20	AAAGAGGCCG	AGTTCAAGGC	AATGCTGGAG	CAACTCAAGA	AGCAGCAGGA	1499
	GGAGGCACAG	GCTGCTGCAA	TGGCCACCTC	AGCGGGCACT	GTCTCGGAAG	1549
	ACGCCATTGA	AGAAGAAGGG	GAAGATGGGG	TAGGCTCTCC	GAGGAGCTCT	1599
	TCTGAACTGT	CTAAACTCAG	TTCCAAGAGC	GCGAAGGAGC	GGCGGAACCG	1649
	ACGGAAGAAG	AGGAAGCAGA	AGGAGCTCTC	TGAAGGCGAG	GAGAAAGGGG	1699
25	ACCCGGAGAA	GGTGTTTAAG	TCAGAGTCGG	AAGACGGTAT	GAGAAGGAAG	1749
	GCCTTCCGGC	TGCCAGACAA	CAGGATAGGG	AGGAAGTTTT	CCATCATGAA	1799
	TCAGTCGCTG	CTCAGCATTC	CAGGCTCGCC	CTTCCTCTCC	CGACATAACA	1849
	GCAAAAGCAG	CATCTTCAGC	TTCCGGGGAC	CCGGTCGGTT	CCGGGACCCC	1899
	GGCTCTGAGA	ATGAGTTCGC	AGACGATGAA	CACAGCACCG	TGGAGGAGAG	1949
30	CGAGGGCCCG	CGTGACTCGC	TCTTCATCCC	GATCCGCGCC	CGCGAGCGCC	1999
	GCAGCAGCTA	CAGTGGCTAC	AGCGGCTACA	GCCAGTGCAG	CCGCTCGTCG	2049
	CGCATCTTCC	CCAGCCTGCG	GCGCAGCGTG	AAGCGCAACA	GCACGGTGGA	2099
	CTGCAACGGC	GTAGTGTCAC	TCATCGGGCC	CGGCTCACAC	ATCGGGCGGC	2149
	TCCTGCCTGA	GGCAACGACT	GAGGTGGAAA	TTAAGAAGAA	AGGCCCTGGA	2199
35	TCTCTTTTAG	TTTCTATGGA	CCAACTCGCC	TCCTACGGAC	GGAAGGACAG	2249
	AATCAACAGC	ATAATGAGCG	TGGTCACAAA	CACGCTAGTG	GAAGAGCTGG	2299

	AAGAGTCTCA	GAGAAAGTGC	CCACCGTGCT	GGTATAAGTT	TGCCAACACT	2349
	TTCCCTCATCT	GGGAGTGTCA	CCCCTACTGG	ATAAACTGA	AGGAGATCGT	2399
	GAACCTTAATC	GTCATGGACC	CTTTTGTAGA	CTTAGCCATC	ACCATCTGCA	2449
	TCGTTCTGAA	TACGCTATTT	ATGGCAATGG	AGCACCATCC	CATGACACCA	2499
5	CAGTTCGAAC	ACGTCTTGGC	CGTAGGAAAT	CTGGTGTTCA	CCGGGATCTT	2549
	CACGGCGGAA	ATGTTTCTGA	AGCTCATAGC	CATGGACCCC	TACTATTATT	2599
	TCCAAGAAGG	CTGGAACATT	TTTGACGGAT	TTATTGTCTC	CCTCAGTTTA	2649
	ATGGAGCTGA	GTCTCGCAGA	TGTGGAGGGG	CTCTCAGTGC	TGCGGTCTTT	2699
	CCGACTGCTC	CGAGTCTTCA	AGCTGGCCAA	GTCCTGGCCC	ACCCTGAACA	2749
10	TGCTGATCAA	GATCATCGGG	AACTCCGTGG	GTGCCCTGGG	CAACCTGACC	2799
	CTGGTGCTGG	CCATCATCGT	CTTCATCTTC	GCCGTGGTGG	GGATGCAGCT	2849
	GTTTGGAAG	AGTTACAAGG	AGTGCCTCTG	TAAGATCAAC	CAGGAGTGCA	2899
	AGCTCCCGCG	CTGGCACATG	AACGACTTCT	TCCACTCCTT	CCTCATCGTC	2949
	TTCCGAGTGC	TGTGTGGGGA	GTGGATCGAG	ACCATGTGGG	ACTGCATGGA	2999
15	GGTGGCCGGC	CAGGCCATGT	GCCTCATTGT	CTTCATGATG	GTTATGGTCA	3049
	TTGGCAACCT	GGTGGTGCTG	AATCTATTCC	TGGCCTTGCT	TCTGAGCTCC	3099
	TTCAGCGCAG	ACAACCTGGC	GGCCACAGAC	GACGACGGGG	AAATGAACAA	3149
	CCTGCAGATC	TCAGTGATCC	GGATCAAGAA	GGGCGTGGCC	TGGACCAAAG	3199
	TGAAGGTGCA	CGCCTTCATG	CAGGCTCACT	TCAAGCAGCG	GGAGGCGGAT	3249
20	GAAGTGAAAC	CCCTCGACGA	GCTGTATGAG	AAGAAGGCCA	ACTGCATCGC	3299
	CAACCACACG	GGCGTGGATA	TCCACCGGAA	CGGCGACTTC	CAGAAGAACG	3349
	GGAACGGAAC	CACCAGCGGC	ATCGGCAGCA	GCGTGGAGAA	GTACATCATC	3399
	GACGAGGACC	ACATGTCCTT	CATTAACAAC	CCAAACCTGA	CCGTCCGGGT	3449
	GCCCATTGCT	GTGGGCGAGT	CTGACTTCGA	GAACCTCAAC	ACAGAGGATG	3499
25	TTAGCAGCGA	ATCAGACCTT	GAAGGCAGCA	AAGATAAACT	GGACGATACC	3549
	AGCTCCTCAG	AAGGAAGTAC	CATCGACATC	AAGCCTGAGG	TGGAAGAAGT	3599
	TCCCGTGGAG	CAACCTGAGG	AATACTTGGA	TCCGGACGCC	TGCTTTACAG	3649
	AGGGTTGCGT	CCAGCGGTTC	AAGTGCTGCC	AGGTCAACAT	CGAGGAAGGA	3699
	CTAGGCAAGT	CGTGGTGGAT	CTTGCGGAAA	ACCTGCTTCC	TCATTGTGGA	3749
30	GCACAATTGG	TTTGAGACCT	TCATCATCTT	CATGATTCTG	CTCAGCAGTG	3799
	GCGCCCTGGC	CTTTGAGGAC	ATCTACATTG	AGCAGAGGAA	GACCATCCGC	3849
	ACCATCCTGG	AGTATGCGGA	CAAGGTCTTC	ACCTACATCT	TCATCCTGGA	3899
	GATGTTGCTC	AAGTGGACAG	CCTACGGCTT	CGTCAAGTTC	TTCACCAATG	3949
	CCTGGTGCTG	GTTGGACTTC	CTCATTGTGG	CTGTCTCTTT	AGTCAGCCTT	3999
35	ATAGCTAATG	CCCTGGGCTA	CTCGGAATA	GGTGCCATAA	AGTCCCTTAG	4049
	GACCCTAAGA	GCTTTGAGAC	CCTTAAGAGC	CTTATCACGA	TTTGAAGGGA	4099

	TGAGGGTGGT	GGTGAATGCC	TTGGTGGGCG	CCATCCCCTC	CATCATGAAT	4149
	GTGCTGCTGG	TGTGTCTCAT	CTTCTGGCTG	ATTTTCAGCA	TCATGGGAGT	4199
	TAACCTGTTT	GCGGGGAAAT	ACCACTACTG	CTTTAATGAG	ACTTCTGAAA	4249
	TCCGGTTCGA	AATCGATATT	GTCAACAATA	AAACGGACTG	TGAGAAGCTC	4299
5	ATGGAGGGCA	ACAGCACGGA	GATCCGATGG	AAGAATGTCA	AGATCAACTT	4349
	TGACAATGTC	GGAGCAGGGT	ACCTGGCCCT	TCTTCAAGTG	GCAACCTTCA	4399
	AAGGCTGGAT	GGACATCATG	TATGCGGCTG	TAGATTCCCG	AAAGCCAGAC	4449
	GAGCAGCCTG	ACTACGAGGG	CAACATCTAC	ATGTACATCT	ACTTCGTCAT	4499
	CTTCATCATC	TTCGGCTCCT	TCTTCACCCT	CAACCTGTTC	ATCGGTGTCA	4549
10	TCATCGACAA	CTTCAACCAG	CAGAAGAAAA	AGTTTGGAGG	TCAGGACATC	4599
	TTCATGACAG	AGGAACAGAA	GAAGTACTAC	AATGCCATGA	AAAAGCTGGG	4649
	CTCCAAGAAG	CCACAGAAGC	CCATCCCCCG	ACCCTTGAAC	AAAATCCAAG	4699
	GGATTGTCTT	TGATTTCGTC	ACTCAACAAG	CCTTTGACAT	TGTGATCATG	4749
	ATGCTCATCT	GCCTTAACAT	GGTGACAATG	ATGGTGGAGA	CAGACACTCA	4799
15	GAGCAAGCAG	ATGGAGAACA	TTCTTTACTG	GATTAATCTG	GTCTTTGTCA	4849
	TCTTCTTCAC	CTGCGAGTGT	GTGCTCAAAA	TGTTTGCCTT	GAGACACTAC	4899
	TATTTACCA	TTGGCTGGAA	CATCTTTGAC	TTTGTGGTGG	TCATCCTCTC	4949
	CATTGTGGGA	ATGTTCTGG	CTGATATCAT	TGAGAAGTAC	TTCGTCTCCC	4999
	CAACCCTATT	CCGAGTTATC	CGATTGGCCC	GTATTGGGCG	CATCTTGCGT	5049
20	CTGATCAAGG	GCGCCAAAGG	GATCCGCACC	CTGCTCTTTG	CCTTAATGAT	5099
	GTCGCTGCCC	GCCCTGTTCA	ACATCGGCCT	CCTGCTCTTC	CTCGTCATGT	5149
	TCATCTTCTC	CATTTTTGGC	ATGTCCAAC	TCGCATACGT	GAAGCACGAG	5199
	GCCGGCATTG	ACGACATGTT	CAACTTCGAG	ACATTTGGCA	ACAGCATGAT	5249
	CTGTTTGTTC	CAGATCACAA	CGTCTGCTGG	CTGGGATGGC	CTGCTGCTGC	5299
25	CAATCCTGAA	CCGCCCCCT	GACTGCAGCT	TGGACAAAGA	GCACCCAGGG	5349
	AGTGGCTTCA	AAGGGGACTG	TGGGAACCCC	TCGGTGGGCA	TCTTCTTCTT	5399
	TGTGAGCTAC	ATCATCATCT	CCTTCCTGAT	TGTGGTGAAC	ATGTACATCG	5449
	CCATCATCCT	GGAGAACTTC	AGCGTGGCCA	CCGAGGAGAG	CGCCGACCCT	5499
	CTGAGTGAGG	ATGACTTCGA	GACTTTCTAT	GAGATCTGGG	AGAAGTTTGA	5549
30	CCCAGACGCC	ACCCAGTTCA	TCGAGTACTG	TAAGCTGGCA	GACTTTGCCG	5599
	ACGCCCTGGA	GCACCCGCTC	CGAGTACCCA	AGCCCAACAC	CATCGAGCTC	5649
	ATCGCCATGG	ACCTGCCCAT	GGTGAGCGGA	GATCGCATCC	ACTGCTTGGA	5699
	CATCCTTTTC	GCCTTCACCA	AGCGAGTCCT	GGGAGACAGT	GGGGAGTTGG	5749
	ACATCCTGCG	GCAGCAGATG	GAGGAGCGGT	TCGTGGCATC	CAATCCTTCC	5799
35	AAAGTGTCTT	ACGA-GCCTA	TCACAACCAC	TCTGCGGCGC	AAGCAGGAGG	5848
	AGGTGTCTGC	AGTGGTCCTG	CAGCGTGCCT	ACAGGGGACA	CTTGGCTAGG	5898

	CGGGGCTTCA	TCTGCAGAAA	GATGGCCTCC	AACAAGCTGG	AGAATGGAGG	5948
	CACACACAGA	GACAAGAAGG	AGAGCACCCC	GTCCACAGCC	TCCCTCCCCCT	5998
	CTTACGACAG	CGTCACAAAG	CCAGACAAGG	AGAAGCAGCA	GCGTGCGGAG	6048
	GAGGGCAGAA	GGGAAAGAGC	CAAGAGGCAA	AAAGAGGTCA	GGGAGTCCAA	6098
5	GTGCTAGAGG	AGGGGAAAGG	AAGCTTACCC	CGGCTGAACA	CTGGCAAGTG	6148
	AAAGCTTGTT	TACAAACTTC	CGAATCTCAC	GGATGCAGAG	CAGCTGTGCA	6198
	GACGCTCGCT	GTACTGGAAG	ACCTATACCA	AACATAGTCT	GCTTACATGT	6248
	GACATGGTGG	CATCCTGAGC	GGTGA---CT	GCTGGGGACA	AAGGACCCTG	6295
	CTCCCTGGAC	TCACAGATCT	CCTATCGCTT	GGGCAGACGG	TTACTGCATG	6345
10	TTCCCACTT	AGTCAATGCA	ACTTAGGACT	AAACTAACCA	GGATACAAAA	6395
	CCGAGGCGGC	TGCCGGGACC	AGCAGATCAC	CGCTGCAGCC	AAATGGATTT	6445
	TATTTTTTCA	TTTGTTGAT	TCTCAGAAGC	AGAAAGCATC	ACTTTAAAAG	6495
	TTTGTTTGTT	CATNCAAACA	ATATTTGAAT	TCTTACATTA	GTTAAGCTAA	6545
	GCANCAAAAA	G-----	-----	-----	-----	6556

Claims

1. An isolated DNA sequence comprising the nucleotide sequence set forth in SEQ ID NO:7.
5
2. The isolated DNA sequence of claim 1 comprising the nucleotide sequence as set forth in SEQ ID NO:1.
3. The DNA of Claim 2 wherein said DNA sequence is encoding a sodium
10 channel protein.
4. The DNA of Claim 3 wherein said sodium channel protein is the α -subunit.
5. The DNA of Claim 4 wherein said sodium channel protein is tetrodotoxin-
15 sensitive.
6. The DNA of Claim 5 wherein said sodium channel protein is found in mammals.
7. The DNA of Claim 5 wherein said sodium channel protein is found in rat.
20
8. The DNA of Claim 5 wherein said sodium channel protein is found in human.
9. The DNA of Claim 2 wherein said DNA is cDNA.
25
10. The DNA of Claim 2 wherein said DNA is synthetic DNA.
11. Expression vectors comprising a DNA as claimed in claims 2 to 10.
30
12. Host cells transformed with an expression vector of Claim 11.
13. A cDNA library comprising a host cell of Claim 12.
14. A recombinant polynucleotide comprising a nucleic acid sequence derived
35 from a DNA sequence as claimed in claims 2 to 10.

15. A tetrodotoxin-sensitive sodium channel protein encoded by a DNA of claims 2 to 10 or allelic variants thereof.
- 5 16. The protein having the amino acid sequence set forth in SEQ ID NO:3.
17. An assay for inhibitors of tetrodotoxin-sensitive sodium channel protein encoded by a DNA of claims 2 to 10 or the amino acid sequence as set forth in SEQ ID NO:3 comprising contacting a compound suspected of being said inhibitor with expressed
10 sodium channel protein and measuring the activity of said expressed sodium channel protein.
18. An isolated DNA sequence comprising the nucleotide sequence set forth in SEQ ID NO:7 having a 30 base pair insert after base pair number 2050, the 30 base pair
15 insert comprising GTGAAAATAGATAAGGCAGCTACGGACAGC.
19. The isolated DNA sequence of claim 18 comprising the nucleotide sequence as set forth in SEQ ID NO:2.
- 20 20. The DNA of Claim 19 wherein said DNA sequence is encoding a sodium channel protein.
21. The DNA of Claim 20 wherein said sodium channel protein is the α -
subunit.
25
22. The DNA of Claim 21 wherein said sodium channel protein is tetrodotoxin-sensitive.
23. The DNA of Claim 22 wherein said sodium channel protein is found in
30 mammal.
24. The DNA of Claim 22 wherein said sodium channel protein is found in rat.
25. The DNA of Claim 22 wherein said sodium channel protein is found in
35 human.

26. The DNA of Claim 19 wherein said DNA is cDNA.
27. The DNA of Claim 19 wherein said DNA is synthetic DNA.
- 5 28. Expression vectors comprising a DNA as claimed in claims 19 to 27.
29. Host cells transformed with an expression vector of Claim 28.
30. A cDNA library comprising a host cell of Claim 29.
- 10 31. A recombinant polynucleotide comprising a nucleic acid sequence derived from a DNA sequence as claimed in claims 19 to 27.
32. A tetrodotoxin-sensitive sodium channel protein encoded by a DNA of
- 15 Claims 19 to 27 or allelic variants thereof.
33. The protein having the amino acid sequence set forth in SEQ ID NO:4.
34. An assay for inhibitors of tetrodotoxin-sensitive sodium channel protein
- 20 encoded by a DNA of claims 19 to 27 or the amino acid sequence as set forth in SEQ ID NO:4 comprising contacting a compound suspected of being said inhibitor with expressed sodium channel protein and measuring the activity of said expressed sodium channel protein.
- 25 35. An isolated polynucleotide probe comprising the nucleotide sequence set forth in SEQ ID NO:5 and complements thereof.
36. The protein having the amino acid sequence set forth in SEQ ID NO:6.
- 30 37. A polynucleotide probe comprising the polynucleotide of Claim 35 bound to a reporter molecule.
38. A method of growing plasmids containing constructs of tetrodotoxin-sensitive sodium channel proteins comprising:
- 35

employing competent *E. coli* cell lines for primary transformations which allow the stable propagation of plasmids containing unstable inserts following ligation reactions;

employing 1/2 strength freezing media mixed with a broth chosen from the group comprising full strength YENB, full strength YET, and full strength LB, the broth being
5 mixed with agar for solid media;

employing full strength freezing media plus 1/2 strength LB for liquid media;

employing carbenicillin as an antibiotic;

providing a temperature no greater than 30° C; and

growing the plasmids for periods longer than 20 hours.

10

39. The method according to claim 38 employing competent *E. coli* cells for secondary transformants.

15

40. The method according to claim 38 wherein the temperature is 28° C.

41. The method according to claim 38 wherein the growing period is in the range of approximately 36 to 48 hours.

20

42. The method according to claim 38 having a carbenicillin concentration within the range of 50-200 µg/ml.

43. The method according to claim 38 wherein the carbenicillin has a concentration within the range of 75-125 µg/ml.

25

44. The method according to claim 38 wherein the carbenicillin has a concentration of 100 µg/ml.

45. Antibodies against a tetrodotoxin-sensitive sodium channel protein as claimed in claims 15, 16, 32 and 33.

30

46. The use of a tetrodotoxin-sensitive sodium channel protein as claimed in claims 15, 16, 32 and 33 for identifying inhibitors of their activity.

47. A method of producing a tetrodotoxin-sensitive sodium channel protein as
35 claimed in claims 15, 16, 32 and 33, comprising cultivating a host cell as claimed in claim 12 or 29 in a suitable medium and optionally isolating said channel protein.

48. Proteins as claimed in claims 15, 16, 32 and 33 prepared by the method of claim 47.

5 49. Proteins as claimed in claims 15, 16, 32 and 33 for identifying inhibitors of their activity.

50. The invention substantially as hereinbefore described, especially with reference to the examples.

10

Fig. 1A: SEQ ID NO:3

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1  MAARLLAPPG PDSFKPFTPE SLANIERRIA ESKLKKPPKA DGSHREDDDED
51  SKPKPNSDLE AGKSLPFIYG DIPQGLVAVP LEDFDPYYLT QKTFVVLNRG
101 KTLFRFSATP ALYILSPFNL IRRIAIIKILI HSFVFSMIIMC TILTNCVFMT
      |-----IS1-----|
151 FSNPPPEWSKN VEYTFGTGIYT FESLVKIIAR GFCIDGFTFL RDPWNWLDFS
      |-----IS2-----| |-----
201 VIMMAYVTEF VDLGNVSALR TFRVLRLAKT ISVIPGLKTI VGALIQSVKK
      IS3-----| * |-----IS4-----|
251 LSDVMILTVE CLSVFALIGL QLFMGNLRNK CVVWPINFNE SYLENGTRGF
      *|-----IS5-----| * *
301 DWEEYINNKT NFYMVPGMLE PLLCGNSSDA GQCPEGFQCM KAGRNPNYGY
      * *
351 TSFDTFSWAF LALFRLMTQD YWENLYQLTL RAAGKTYMIF FVLVIFVGSF
      Δ |-----IS6-
401 YLVNLILAVV AMAYEEQNQA TLEEAEQKEA EFKAMLEQLK KQEEAQAAA
      -----|
451 MATSAGTVSE DAIEEEGEDG VGSPRSSSEL SKLSSKSAKE RRNRKRKRKQ
501 KELSEGEEKG DPEKVFSES EDGMRRKAFR LPDNRIGRKF SIMNQSLLSI
      * *
551 PGSPFLSRHN SKSSIFSFRG PGRFRDPGSE NEFADDEHST VEESEGRDS
      *
601 LFIPIRARER RSSYSGYSGY SQCSRSSRIF PSLRRSVKRN STVDCNGVVS
      * *
651 LIGPGSHIGR LLPEATTEVE IKKKGPGSLL VSMDQLASYG RKDRINSIMS
701 VVTNTLVEEL EESQRKCPPC WYKFANTFLI WECHPYWIKL KEIVNLIVMD
      |-----
751 PFVDLAITIC IVLNTLFMAM EHHPMTPQFE HVLAVGNLVF TGIFTAEMFL
      ----IIS1-----| |-----IIS2-----
801 KLIAMDPIYY FQEGWNIFDG FIVSLSLMEL SLADVEGLSV LRSFRLLRVF
      ----| |-----IIS3-----| |-----IIS4

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2/23

Fig. 1B: SEQ ID NO:3

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351  KLAWSWPTLN MLIKIIGNSV GALGNLTLVL AIIVFIFAVV GMQLFGKSYK
      -----|               ♦ |-----IISS-----|
901  ECVCKINQEC KLPRWHMND FHSFLIVFRV LCGEWIETMW DCMEVAGQAM
                                     |-
951  CLIVFMMVMV IGNLVVLNLF LALLSSFSFA DNLAATDDD GEMNNLQISVI
      -----IIS6-----|
1001  RIKKGVAVTK VKVHAFMQAH FKQREADEVK PLDELYEKKA NCIANHTGVD
                                     ♦
1051  IHRNGDFQKN GNGTTSGIGS SVEKYIDED HNSFINNP NL TVRVPIAVGE
      ♦                               ♦
1101  SDFENLNTED VSSSDPEGS KDKLDDTSSS EGSTIDIKPE VEEVPVEQPE
1151  EYLDPDACFT EGCVRQFKCC QVNIEEGLGK SWWILRKTCF LIVEHNWFET
                                     |-----
1201  FIIFMILLSS GALAFEDIYI EQRKTIRTIL EYADKVFTYI FILEMLLKWT
      ---IIIS1-----|               |-----IIIS2-----
1251  AYGFVKFFTN AWCWLDLIV AVSLVSLIAN ALGYSELGAI KSLRTLRLALR
      --|               |-----IIIS3-----|               |-----IIIS4-
1301  PLRLSRFEG MRVVVNALVG AIPSIMNVLL VCLIFWLIFS IMGVNLFAGK
      -----|               |-----IIIS5-----|
1351  YHYCFNETSE IRFEIDIVNN KTDCEKLMEG NSTEIRWKNV KINFDNVGAG
      ♦               ♦               ♦
1401  YLALLQVATF KGWMDIMYAA VDSRKPD EQP DYEGNIYMYI YFVIFIIFGS
                                     |-----IIIS6
1451  FFTLNLFIVG IIDNFNQKK KFGGQDIFMT EEQKKYYNAM KKLGSKKPQK
      -----|
1501  PIPRPLNKIQ GIVFDFVTQQ AFDIVIMMLI CLNMVTMMVE TDTQSKQMEN
                                     |-----IVS1-----|
1551  ILYWINLVFV IFFTCECVLK MFALRHYYFT IGWNIFDFVV VILSIVGMFL
      |-----IVS2-----|               |-----IVS3--
1601  ADIIEKYFVS PTLFRVIRLA RIGRILRLIK GAKGIRTLLE ALMMSLPALF
      ----|               |-----IVS4-----|
1651  NIGLLLFLVM FIFSIFGMSN FAYVKHEAGI DDMFNFETFG NSMICLFQIT
      |-----IVS5-----|

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3/23

Fig. 1C: SEQ ID NO:3

1701 TSAGWDGLLL PILNRPPDCS LDKEHPGSGF KGDCGNPSVG IFFFVSYIII
|-----
1751 SFLIVVNMYI AIILENFSVA TEESADPLSE DDFETFYEIW EKFDPDATQF
IVS6-----| ♦
1801 IEYCKLADFA DALEHPLRVP KPNTIELIAM DLPMVSGDRI HCLDILFAFT
1851 KRVLGDSGEL DILRQOMEER FVASNPSKVS YEPITTTLRR KQEEVSAVVL
1901 QRAYRGHLAR RGFICRKMAS NKLENGGTHR DKKESTPSTA SLPSYDSVTK
1951 PDKEKQQRAE EGRRERAKRQ KEVRESKC

4/23

Fig. 2A: SEQ ID NO:4

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1  MAARLLAAPPG PDSFKPFTPE SLANTIERRIA ESKLKKPPKA DGSHREDDDED
51  SKPKPNSDLE AGKSLPFIYG DIPQGLVAVP LEDFDPYYLT QKTFVVLNRG
101 KTLFRFSATP ALYILSPFNL IRRIAIKILI HSVFSMIIMC TILTNCVFMT
      |-----IS1-----|
151 FSNPPEWSKN VEYFTGTIYT FESLVKIIAR GFCIDGFTFL RDPWNWLDFS
      |-----IS2-----|           |-----
201 VIMMAYVTEF VDLGNVSALR TFRVLRLAKT ISVIPGLKTI VGALIQSVKK
      IS3-----|   ♦   |-----IS4-----|
251 LSDVMILTVE CLSVFALIGL QLFMGNLRNK CVVWPINFNE SYLENGTRGF
      ♦|-----IS5-----|           ♦   ♦
301 DWEEYINNKT NFYMVPGMLE PLLCGNSSDA GQCEGFQCM KAGRNPNYGY
      ♦           ♦
351 TSFDTFSWAF LALFRLMTQD YWENLYQLTL RAAGKTYMIF FVLVIFVGSF
      Δ           |-----IS6
401 YLVNLILAVV AMAYEEQNQA TLEEAEQKEA EFKAMLEQLK KQEEEAQAAA
      -----|
451 MATSAGTVSE DAIEEEGEDG VGSPRSSSEL SKLSSXSAKE RPNRRKKRKQ
501 KELSEGEEKG DPEKVPKSES EDGMRRKAFR LPDNRIGRKF SIMNQSLLSI
      ♦ ♦
551 PGSPFLSRHN SKSSIFSFRG PGRFRDPGSE NEFADDEHST VEESEGRDSD
      ♦
601 LFIPIRERER RSSYSGYSGY SQCSRSSRIF PSLRRSVKRN STVDCNGVVS
      ♦ ♦
651 LIGPGSHIGR LLPEVKIDKA ATDSATTEVE IKKKGPGSLL VSMDQLASYG
701 RKDRINSIMS VVTNTLVEEL EESQRKCPPC WYKFANTFLI WECHPYWIKL
751 KEIVNLIVMD PFVDLAITIC IVLNTLFMAM EHHPMTPQFE HVLAVGNLVE
      |-----IIS1-----|           |-----
801 TGIFTAEMFL KLIAMPYYY FQEGWNIFDG FIVSLSLMEL SLADVEGLSV
      IIS2-----|           |-----IIS3-----|

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5/23

Fig. 2B: SEQ ID NO:4

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851  LRSFRLLRVF KLAKSWPTLN MLIKIIGNSV GALGNLTLVL AIIVFIFAVV
      -----IIS4-----|                               ♦|-----IIS5-
901  GMQLFGKSYK ECVCKINQEC KLPRWHMND FHSFLIVFRV LCGEWIETMW
      -----|
951  DCMEVAGQAM CLIVFMMVMV IGNLVVLNLF LALLLSSFSA DNLAATDDDG
      |-----IIS6-----|
1001 EMNQLQISVI RIKKGVAVTK VKVHAFMQAH FKQREAEVK PLDELYEKKA
1051 NCIANHTGVD IHRNGDFQKN GNGTTSIGS SVEKYIIDED HMSFINNPNI
      ♦                               ♦                               ♦
1101 TVRVPIAVGE SDFENLNTED VSSESDEGS KDKLDDTSSS EGSTIDIKPE
1151 VEEVPVEQPE EYLDPDACFT EGCVRQFKCC QVNIEEGLGK SWWILRKTCF
1201 LIVEHNWFET FIIFMILLSS GALAFEDIYI EQRKTIRTIL EYADKVFTYI
      |-----IIS1-----|                               |-----
1251 FILEMLLKWT AYGFVKFFTN AWCWLDFLIV AVSLVSLIAN ALGYSELGAI
      IIS2-----|                               |-----IIS3-----|  |--
1301 KSLRTLRLR PLRALSREFG MRVVVNALVG AIPSIMNVLL VCLIFWLIFS
      -----IIS4-----|                               |-----IIS5
1351 IMGVNLFAGK YHYCFNETSE IRFEIDIVNN KTDCEKLMG NSTEIRWKQV
      -----|                               ♦                               ♦
1401 KINFNVGAG YLALLQVATF KGWMIMYAA VDSRKPDEQP DYEGNIYMYI
      |-----|                               ♦                               |---
1451 YFVIFIIFGS FFTLNLFIV IIDNFNQKK KFGGQDIFMT EEQKKYYNAM
      -----IIS6-----|
1501 KKLGSKKPQK PIPRPLNKIQ GIVDFVTTQ AFDIVIMMLI CLNMVTMMVE
      |-----IVS1-----|
1551 TDTQSKQMEN ILYWINLVFV IFFTCECVLK MFALRHYYFT IGWNIFDFV
      |-----IVS2-----|                               |-----
1601 VILSIVGMFL ADIIEKYFVS PTLFRVIRLA RIGRILRLIK GAKGIRTLF
      ---IVS3-----|                               |-----IVS4-----|
1651 ALMMSLPALF NIGLLLFLVM FIFSIFGMSN FAYVKHEAGI DDMFNFETFG
      |-----IVS5-----|

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6/23

Fig. 2C: SEQ ID NO:4

1701 NSMICLFQIT TSAGWDGLLL PILNRPPDCS LDKEHPGSGF KGDCGNPSVG
|-
1751 IFFVSYIII SFLIVVNMYI AIILENFSVA TEESADPLSE DDFETFYEIW
-----IVS6-----|♦
1801 EKFDPDATQF IEYCKLADFA DALEHPLRVP KPNTIELIAM DLPMVSGDRI

1851 HCLDILFAFT KRVLGDSGEL DILRQQMEER FVASNPSKVS YEPITTTLR

1901 KQEEVSAVVL QRAYRGHLAR RGFICRKMAS NKLENGGTHR DKKESTPSTA
.
1951 SLPSYDSVTK PDKEKQORAE EGRRERAKRQ KEVRESKC

7/23

Fig. 3A: NaCh6/PN4 alignment (SEQ ID NO:7)

RATNaCh6A rPN4	-----	-----	-----	-----	-----	
	CCAAGATGGC	GCCCACCGCA	GTCCCGCCCG	CCGCAGCCTC	GGCGCCTCTG	50
RATNaCh6A rPN4	-----	-----	-----	-----	-----	
	CAGTCCGGCC	GCGCCTCCCG	GGCCCCGCGC	TAGGGCCGCT	GCCGCCTCGC	100
RATNaCh6A rPN4	-----	-----	-----	-----	-----	
	CCGCCGCCGC	CGCCGCCAGC	TGACCTGTCC	CGGACACATA	ACTAACGAAG	150
RATNaCh6A rPN4	-----	start=>	-----	-----	-----	
	CTGCTGCAGG	ATGAGAAGAT	--CGGCGCGG	CTGCTCGCAC	CACCAGGCCC	38
		ATGAGAAGAT	GGCAGCGCGG	CTGCTCGCAC	CACCAGGCCC	200
		start=>	-----	-----	-----	
RATNaCh6A rPN4	TGATAGTTTC	AAGCCTTTCA	CCCCTGAGTC	GCTGGCAAAC	ATCGAGAGGC	88
	TGATAGTTTC	AAGCCTTTCA	CCCCTGAGTC	GCTGGCAAAC	ATCGAGAGGC	250
RATNaCh6A rPN4	GTATTGCCGA	GAGCAAGCTC	AAGAAACCAC	CAAAGGCGGA	TGGCAGCCAC	138
	GTATTGCCGA	GAGCAAGCTC	AAGAAACCAC	CAAAGGCGGA	TGGCAGCCAC	300
RATNaCh6A rPN4	CGGGAGGACG	ATGAAGACAG	CAAGCCCAAG	CCAAACAGTG	ACCTGGAGGC	188
	CGGGAGGACG	ATGAAGACAG	CAAGCCCAAG	CCAAACAGTG	ACCTGGAGGC	350
RATNaCh6A rPN4	TGGGAAGAGT	TTGCCTTTCA	TCTACGGGGA	CATCCCGCAA	GGCCTGGTTG	238
	TGGGAAGAGT	TTGCCTTTCA	TCTACGGGGA	CATCCCGCAA	GGCCTGGTTG	400
RATNaCh6A rPN4	CGGTTCCCCT	GGAGGACTTT	GACCCTTACT	ATTTGACGCA	GAAAACCTTT	298
	CGGTTCCCCT	GGAGGACTTT	GACCCTTACT	ATTTGACGCA	GAAAACCTTT	450
RATNaCh6A rPN4	GTAGTATTAA	ACAGAGGGAA	AACCTCTCTC	AGATTTAGTG	CCACACCTGC	338
	GTAGTATTAA	ACAGAGGGAA	AACCTCTCTC	AGATTTAGTG	CCACACCTGC	500
RATNaCh6A rPN4	CTTGACATT	TTAAGCCCTT	TTAACCTGAT	AAGAAGAATA	GCTATTAAAA	388
	CTTGACATT	TTAAGCCCTT	TTAACCTGAT	AAGAAGAATA	GCTATTAAAA	550
RATNaCh6A rPN4	TTTTGATACA	CTCAGTTTTT	AGCATGATCA	TCATGTGCAC	CATCCTGACC	438
	TTTTGATACA	CTCAGTTTTT	AGCATGATCA	TCATGTGCAC	CATCCTGACC	600
RATNaCh6A rPN4	AACCTGTGTG	TCATGACCTT	TAGTAACCCT	CCAGAATGGT	CCAAGAATGT	488
	AACCTGTGTG	TCATGACCTT	TAGTAACCCT	CCAGAATGGT	CCAAGAATGT	650
RATNaCh6A rPN4	GGAGTACACA	TTCACAGGGA	TTTACACATT	TGAATCACTA	GTGAAAATCA	538
	GGAGTACACA	TTCACAGGGA	TTTACACATT	TGAATCACTA	GTGAAAATCA	700
RATNaCh6A rPN4	TCGCAAGAGG	TTTCTGCATA	GACGGCTTCA	CCTTCTTACG	AGACCCGTGG	588
	TCGCAAGAGG	TTTCTGCATA	GACGGCTTCA	CCTTCTTACG	AGACCCGTGG	750
RATNaCh6A rPN4	AACCTGGTTAG	ACTTCAGTGT	CATCATGATG	GCAATATGTGA	CAGAGTTTGT	638
	AACCTGGTTAG	ACTTCAGTGT	CATCATGATG	GCAATATGTGA	CAGAGTTTGT	800
RATNaCh6A rPN4	GGACCTGGGC	AATGTCTCAG	CGCTGAGAAC	ATTCAGGGTT	CTCCGAGCTT	688
	GGACCTGGGC	AATGTCTCAG	CGCTGAGAAC	ATTCAGGGTT	CTCCGAGCTT	850

8/23

Fig. 3B: NaCh6/PN4 alignment (SEQ ID NO:7)

RATNaCh6A	TGAAAACTAT	CTCTGTAATT	CCAGGCCTGA	AGACAATCGT	GGGCGCCCTA	738
rPN4	TGAAAACTAT	CTCTGTAATT	CCAGGCCTGA	AGACAATCGT	GGGCGCCCTA	900

	--->					
RATNaCh6A	ATCCAGTCCG	TGAAGAAGCT	GTCGGACGTG	ATGATCCTGA	CAGTGTTCTG	788
rPN4	ATCCAGTCCG	TGAAGAAGCT	GTCGGACGTG	ATGATCCTGA	CAGTGTTCTG	950
RATNaCh6A	CCTGAGTGTT	TTCGCCCTGA	TTGGCCTGCA	GCTCTTTCAT	GGGAACCTTT	838
rPN4	CCTGAGTGTT	TTCGCCCTGA	TTGGCCTGCA	GCTCTTCATG	GGGAACCTT-	999
RATNaCh6A	CGAAAC-AGT	GTGTCGTGTG	GCCCATAAAC	TTCAACGAGA	GCTACCTGGA	887
rPN4	CGAAACAAGT	GTGTCGTGTG	GCCCATAAAC	TTCAACGAGA	GCTACCTGGA	1049
RATNaCh6A	GAACGGCACC	AGAGGCTTTG	ACTGGGAGGA	ATATATCAAC	AATAAAACAA	937
rPN4	GAACGGCACC	AGAGGCTTTG	ACTGGGAGGA	ATATATCAAC	AATAAAACAA	1099
RATNaCh6A	ACTTTTACAT	GGTTCCTGGC	ATGCTAGAAC	CCTTGCTCTG	CGGGAACAGT	987
rPN4	ACTTTTACAT	GGTTCCTGGC	ATGCTAGAAC	CCTTGCTCTG	CGGGAACAGT	1149
	←-----					
RATNaCh6A	TCTGATGCTG	GGCAATGC--	-GAAGGATTC	CAGTGCAGTA	AAGCAGGAAG	1034
rPN4	TCTGATGCTG	GGCAATGCCC	AGAGGGATTC	CAGTGCATGA	AAGCAGGAAG	1199
	←-----					
RATNaCh6A	GAACCCCAAC	TACGGTTACA	CCAGCTTTGA	CACCTTCAGC	TGGGCCTTCT	1084
rPN4	GAACCCCAAC	TACGGTTACA	CCAGCTTTGA	CACCTTCAGC	TGGGCCTTCT	1249
RATNaCh6A	TGGCATTATT	CCGCCTTATG	ACCCAGGACT	ATTGGGAGAA	CTTATACCAG	1134
rPN4	TGGCATTATT	CCGCCTTATG	ACCCAGGACT	ATTGGGAGAA	CTTATACCAG	1299
RATNaCh6A	CTGACCTTAC	GAGCCGCTGG	GAAAACGTAC	ATGATCTTCT	TTGTCTTGGT	1184
rPN4	CTGACCTTAC	GAGCCGCTGG	GAAAACGTAC	ATGATCTTCT	TTGTCTTGGT	1349
RATNaCh6A	CATCTTCGTG	GGTTCTTTCT	ATCCGGTGAA	CTTGATCTTG	GCTGTGGTGG	1234
rPN4	CATCTTCGTG	GGTTCTTTCT	ATCTGGTGAA	CTTGATCTTG	GCTGTGGTGG	1399
RATNaCh6A	CCATGGCTTA	TGAGGAACAG	AACCAGGCAA	CACTGGAGGA	GGCAGAGCAA	1284
rPN4	CCATGGCTTA	TGAGGAACAG	AACCAGGCAA	CACTGGAGGA	GGCAGAGCAA	1449
RATNaCh6A	AAAGAGGCCG	AGTTCAAGGC	AATGCTGGAG	CAACTCAAGA	AGCAGCAGGA	1334
rPN4	AAAGAGGCCG	AGTTCAAGGC	AATGCTGGAG	CAACTCAAGA	AGCAGCAGGA	1499
RATNaCh6A	GGAGGCACAG	GCTGCTGCAA	TGGCCACCTC	AGCGGGCACT	GTCTCGGAAG	1384
rPN4	GGAGGCACAG	GCTGCTGCAA	TGGCCACCTC	AGCGGGCACT	GTCTCGGAAG	1549
RATNaCh6A	ACGCCATTGA	AGAAGAAGGG	GAAGATGGGG	TAGGCTCTCC	GAGGAGCTCT	1434
rPN4	ACGCCATTGA	AGAAGAAGGG	GAAGATGGGG	TAGGCTCTCC	GAGGAGCTCT	1599
RATNaCh6A	TCTGAACTGT	CTAAACTCAG	TTCCAAGAGC	GCGAAGGAGC	GGCGGAACCG	1484
rPN4	TCTGAACTGT	CTAAACTCAG	TTCCAAGAGC	GCGAAGGAGC	GGCGGAACCG	1649
RATNaCh6A	ACGGAAGAAG	AGGAAGCAGA	AGGAGCTCTC	TGAAGGCGAG	GAGAAAGGGG	1534
rPN4	ACGGAAGAAG	AGGAAGCAGA	AGGAGCTCTC	TGAAGGCGAG	GAGAAAGGGG	1599

9/23

Fig. 3C: NaCh6/PN4 alignment (SEQ ID NO:7)

RATNaCh6A	ACCCGGAGAA	GGTGTTTAAG	TCAGAGTCGG	AATACGGTAT	GAGAAGGAAG	1584
rPN4	ACCCGGAGAA	GGTGTTTAAG	TCAGAGTCGG	AAGACGGTAT	GAGAAGGAAG	1749
RATNaCh6A	GCCTTCCGGC	TGCCAGACAA	CAGGATAGGG	AGGAAGTTTT	CCATCATGAA	1634
rPN4	GCCTTCCGGC	TGCCAGACAA	CAGGATAGGG	AGGAAGTTTT	CCATCATGAA	1799
RATNaCh6A	TCAGTCGCTG	CTCAGCATTC	CAGGCTCGCC	CTTCCTCTCC	CGACATAACA	1684
rPN4	TCAGTCGCTG	CTCAGCATTC	CAGGCTCGCC	CTTCCTCTCC	CGACATAACA	1849
RATNaCh6A	GCAAAAGCAG	CATCTTCAGC	TTC-GGGGAC	CC-GTCGGTT	-CGGGACCCC	1731
rPN4	GCAAAAGCAG	CATCTTCAGC	TTCCGGGGAC	CCGGTCGGTT	CCGGGACCCC	1899
RATNaCh6A	GGCTCTGAGA	ATGAGTTTCG	AGACGATGAA	CACAGCACCG	TGGAGGAGAG	1781
rPN4	GGCTCTGAGA	ATGAGTTTCG	AGACGATGAA	CACAGCACCG	TGGAGGAGAG	1949
RATNaCh6A	CGAGGGCCGG	CGTGA CTGC	TCTTCATCCC	GATCCGCGCC	CGCGAGCGCC	1831
rPN4	CGAGGGCCGG	CGTGA CTGC	TCTTCATCCC	GATCCGCGCC	CGCGAGCGCC	1999
RATNaCh6A	GCAGCAGCTA	CAGTGGCTAC	AGCGGCTACA	GCCAGTGCAG	CCGCTCGTCG	1881
rPN4	GCAGCAGCTA	CAGTGGCTAC	AGCGGCTACA	GCCAGTGCAG	CCGCTCGTCG	2049
RATNaCh6A	CGCATCT-CC	CCAGCCTGC-	GCGCAGCGTG	AAGC-CAACA	GCACGGTGGA	1928
rPN4	CGCATCTTCC	CCAGCCTGCG	GCGCAGCGTG	AAGCGCAACA	GCACGGTGGA	2099
RATNaCh6A	CTGCAACGGC	GTAGTGTAC	TCATCGGGCC	CGGCTCACAC	ATCGGGCGGC	1978
rPN4	CTGCAACGGC	GTAGTGTAC	TCATCGGGCC	CGGCTCACAC	ATCGGGCGGC	2149
RATNaCh6A	TCCTGC-TGA	GGCAACGACT	GAGGTGGAAA	TTAAGAAGAA	AGGCCCTGGA	2027
rPN4	TCCTGCCTGA	GGCAACGACT	GAGGTGGAAA	TTAAGAAGAA	AGGCCCTGGA	2199
RATNaCh6A	-CTCTTTTAG	TTTCTATGGA	CCAAC TCGC	TCCTACGGAC	GGAAGGACAG	2076
rPN4	TCTCTTTTAG	TTTCTATGGA	CCAAC TCGC	TCCTACGGAC	GGAAGGACAG	2249
RATNaCh6A	AATCAACAGC	ATAATGAGCG	TGGTCACAAA	CACGCTAGT-	GAAGAGCTGG	2125
rPN4	AATCAACAGC	ATAATGAGCG	TGGTCACAAA	CACGCTAGTG	GAAGAGCTGG	2299
RATNaCh6A	AAGAGTCTCA	GAGAAAGTGC	CCACCGTGCT	GGTATAAGTT	TGCCAACACT	2175
rPN4	AAGAGTCTCA	GAGAAAGTGC	CCACCGTGCT	GGTATAAGTT	TGCCAACACT	2349
RATNaCh6A	TTCCTCATCT	GGGAGTGTCA	CCCCTACTGG	ATAAACTGA	AGGAGATCGT	2225
rPN4	TTCCTCATCT	GGGAGTGTCA	CCCCTACTGG	ATAAACTGA	AGGAGATCGT	2399
RATNaCh6A	GAAC TTAATC	GTCATGGACC	CTTTTGTAGA	CTTAGCCATC	ACCATCTGCA	2275
rPN4	GAAC TTAATC	GTCATGGACC	CTTTTGTAGA	CTTAGCCATC	ACCATCTGCA	2449
RATNaCh6A	TCGTTCTGAA	TACGCTATTT	ATGGCAATGG	AGCACCATCC	CATGACACCA	2325
rPN4	TCGTTCTGAA	TACGCTATTT	ATGGCAATGG	AGCACCATCC	CATGACACCA	2499
RATNaCh6A	CAGTTCGAAC	ACGTCTTGCC	CGTAGGAAAT	CTGGTGTTCA	CCGGGATCTT	2375
rPN4	CAGTTCGAAC	ACGTCTTGCC	CGTAGGAAAT	CTGGTGTTCA	CCGGGATCTT	2549
RATNaCh6A	CACGGCGGAA	ATGTTTCTGA	AGCTCATAGC	CATGGACCCC	TACTATTATT	2425
rPN4	CACGGCGGAA	ATGTTTCTGA	AGCTCATAGC	CATGGACCCC	TACTATTATT	2599

10/23

Fig. 3D: NaCh6/PN4 alignment (SEQ ID NO:7)

RATNaCh6A	TCCAAGAAGG	CTGGAACATT	TTTGACGGAT	TTATTGTCTC	CCTCAGTTTA	2475
rPN4	TCCAAGAAGG	CTGGAACATT	TTTGACGGAT	TTATTGTCTC	CCTCAGTTTA	2649
RATNaCh6A	ATGGAGCTGA	GTCTCGCAGA	TGTGGAGGGG	CTCTCAGTGC	TGCGGTCTTT	2525
rPN4	ATGGAGCTGA	GTCTCGCAGA	TGTGGAGGGG	CTCTCAGTGC	TGCGGTCTTT	2699
RATNaCh6A	CCGACTGCTC	CGAGTCTTCA	AGCTGGCCAA	GTCCTGGCCC	ACCCTGAACA	2575
rPN4	CCGACTGCTC	CGAGTCTTCA	AGCTGGCCAA	GTCCTGGCCC	ACCCTGAACA	2749
RATNaCh6A	TGCTGATCAA	GATCATCGGG	AACTCCGTGG	GTGCCCTGGG	CAACCTGACC	2625
rPN4	TGCTGATCAA	GATCATCGGG	AACTCCGTGG	GTGCCCTGGG	CAACCTGACC	2799
RATNaCh6A	CTGGTGCTGG	CCATCATCGT	CTTCATCTTC	GCCGTGGTGG	GGATGCAGCT	2675
rPN4	CTGGTGCTGG	CCATCATCGT	CTTCATCTTC	GCCGTGGTGG	GGATGCAGCT	2849
RATNaCh6A	GTTTGGAAAG	AGTTACAAGG	AGTGCSTCTG	TAAGATCAAC	CAGGAGTGCA	2725
rPN4	GTTTGGAAAG	AGTTACAAGG	AGTGCSTCTG	TAAGATCAAC	CAGGAGTGCA	2899
RATNaCh6A	AGCTCCCGCG	CTGGCACATG	AACGACTTCT	TCCACTCCTT	CCTCATCGTC	2775
rPN4	AGCTCCCGCG	CTGGCACATG	AACGACTTCT	TCCACTCCTT	CCTCATCGTC	2949
RATNaCh6A	TTCCGAGTGC	TGTGTGGGGA	GTGGATCGAG	ACCATGTGGG	ACTGCATGGA	2825
rPN4	TTCCGAGTGC	TGTGTGGGGA	GTGGATCGAG	ACCATGTGGG	ACTGCATGGA	2999
RATNaCh6A	GGTGGCCGGC	CAGGCCATGT	GCCTCATTGT	CTTCATGATG	GTTATGGTCA	2875
rPN4	GGTGGCCGGC	CAGGCCATGT	GCCTCATTGT	CTTCATGATG	GTTATGGTCA	3049
RATNaCh6A	TTGGCAACCT	GGTGGTGCTG	AATCTATTCC	TGGCCTTGCT	TCTGAGCTCC	2925
rPN4	TTGGCAACCT	GGTGGTGCTG	AATCTATTCC	TGGCCTTGCT	TCTGAGCTCC	3099
RATNaCh6A	TTCAGCGCAG	ACAACCTGGC	GGCCACAGAC	GACGACGGGG	AAATGAACAA	2975
rPN4	TTCAGCGCAG	ACAACCTGGC	GGCCACAGAC	GACGACGGGG	AAATGAACAA	3149
RATNaCh6A	CCTGCAGATC	TCAGTGATCC	GGATCAAGAA	GGGCGTGGCC	TGGACCAAAG	3025
rPN4	CCTGCAGATC	TCAGTGATCC	GGATCAAGAA	GGGCGTGGCC	TGGACCAAAG	3199
RATNaCh6A	TGAAGGTGCA	CGCCTTCATG	CAGGCTCACT	TCAAGCAGCG	GGAGGCGGAT	3075
rPN4	TGAAGGTGCA	CGCCTTCATG	CAGGCTCACT	TCAAGCAGCG	GGAGGCGGAT	3249
RATNaCh6A	GAAGTGAAAC	CCCTCGACGA	GCTGTATGAG	AAGAAGGCCA	ACTGCATCGC	3125
rPN4	GAAGTGAAAC	CCCTCGACGA	GCTGTATGAG	AAGAAGGCCA	ACTGCATCGC	3299
RATNaCh6A	CAACCACACG	GGCGTGGATA	TCCACCGGAA	CGGCGACTTC	CAGAAGAACG	3175
rPN4	CAACCACACG	GGCGTGGATA	TCCACCGGAA	CGGCGACTTC	CAGAAGAACG	3349
RATNaCh6A	GGAACGGAAC	CACCAGCGGC	ATCGGCAGCA	GCGTGGAGAA	GTACATCATC	3225
rPN4	GGAACGGAAC	CACCAGCGGC	ATCGGCAGCA	GCGTGGAGAA	GTACATCATC	3399
RATNaCh6A	GACGAGGACC	ACATGTCCTT	CATTAACAAC	CCAAACCTGA	CCGTCCGGGT	3275
rPN4	GACGAGGACC	ACATGTCCTT	CATTAACAAC	CCAAACCTGA	CCGTCCGGGT	3449
RATNaCh6A	GCCCATTTGCT	GTGGGCGAGT	CTGACTTCGA	GAACCTCAAC	ACAGAGGATG	3325
rPN4	GCCCATTTGCT	GTGGGCGAGT	CTGACTTCGA	GAACCTCAAC	ACAGAGGATG	3499

11/23

Fig. 3E: NaCh6/PN4 alignment (SEQ ID NO:7)

RATNaCh6A	TTAGCAGCGA	ATCAGACCCCT	GAAGGCAGCA	AAGATAAACT	GGACGATACC	3375
rPN4	TTAGCAGCGA	ATCAGACCCCT	GAAGGCAGCA	AAGATAAACT	GGACGATACC	3549
RATNaCh6A	AGCTCCTCAG	AAGGAAGTAC	CATCGACATC	AAGCCTGAGG	TGGAAGAAGT	3425
rPN4	AGCTCCTCAG	AAGGAAGTAC	CATCGACATC	AAGCCTGAGG	TGGAAGAAGT	3599
RATNaCh6A	TCCCGTGGAG	CAACCTGAGG	AATACTTGGA	TCCGGACGCC	TGCTTTACAG	3475
rPN4	TCCCGTGGAG	CAACCTGAGG	AATACTTGGA	TCCGGACGCC	TGCTTTACAG	3649
RATNaCh6A	AGGGTTGCGT	CCAGCGGTTT	AAGTGCTGCC	AGGTCAACAT	CGAGGAAGGA	3525
rPN4	AGGGTTGCGT	CCAGCGGTTT	AAGTGCTGCC	AGGTCAACAT	CGAGGAAGGA	3699
RATNaCh6A	CTAGGCAAGT	CSTGGTGGAT	CTTGCGGAAA	ACCTGCTTCC	TCATTGTGGA	3575
rPN4	CTAGGCAAGT	CSTGGTGGAT	CTTGCGGAAA	ACCTGCTTCC	TCATTGTGGA	3749
RATNaCh6A	GCACAATTGG	TTTGAGACCT	TCATCATCTT	CATGATTCTG	CTCAGCAGTG	3625
rPN4	GCACAATTGG	TTTGAGACCT	TCATCATCTT	CATGATTCTG	CTCAGCAGTG	3799
RATNaCh6A	GCGCCCTGGC	CTTTGAGGAC	ATCTACATTG	AGCAGAGGAA	GACCATCCGC	3675
rPN4	GCGCCCTGGC	CTTTGAGGAC	ATCTACATTG	AGCAGAGGAA	GACCATCCGC	3849
RATNaCh6A	ACCATCCTGG	AGTATGCGGA	CAAGGTCTTC	ACCTACATCT	TCATCCTGGA	3725
rPN4	ACCATCCTGG	AGTATGCGGA	CAAGGTCTTC	ACCTACATCT	TCATCCTGGA	3899
RATNaCh6A	GATGTTGCTC	AAGTGGACCA	CGTACGGCTT	CGTCAAGTTC	TTCACCAATG	3775
rPN4	GATGTTGCTC	AAGTGGACAG	CCTACGGCTT	CGTCAAGTTC	TTCACCAATG	3949
RATNaCh6A	CCTGGTGCTG	GTTGGACTTC	CTCATTGTGG	CTGTCTCTTT	AGTCAGCCTT	3825
rPN4	CCTGGTGCTG	GTTGGACTTC	CTCATTGTGG	CTGTCTCTTT	AGTCAGCCTT	3999
RATNaCh6A	ATAGCTAATG	CCCTGGGCTA	CTCGGAAC TA	GGTGCCATAA	AGTCCCTTAG	3875
rPN4	ATAGCTAATG	CCCTGGGCTA	CTCGGAAC TA	GGTGCCATAA	AGTCCCTTAG	4049
RATNaCh6A	GACCCTAAGA	GCTTTGAGAC	CCTTAAGAGC	CTTATCACGA	TTTGAAGGGA	3925
rPN4	GACCCTAAGA	GCTTTGAGAC	CCTTAAGAGC	CTTATCACGA	TTTGAAGGGA	4099
RATNaCh6A	TGAGGGTGGT	GGTGAATGCC	TTGGTGGGTG	CCATCCCCTC	CATCATGAAT	3975
rPN4	TGAGGGTGGT	GGTGAATGCC	TTGGTGGGCG	CCATCCCCTC	CATCATGAAT	4149
RATNaCh6A	GTGCTGCTGG	TGTGTCTCAT	CTTCTGGCTG	ATTTTCAGCA	TCATGGGAGT	4025
rPN4	GTGCTGCTGG	TGTGTCTCAT	CTTCTGGCTG	ATTTTCAGCA	TCATGGGAGT	4199
RATNaCh6A	TAACCTGTTT	GCGGGGAAAT	ACCACTACTG	CTTTAATGAG	ACTTCTGAAA	4075
rPN4	TAACCTGTTT	GCGGGGAAAT	ACCACTACTG	CTTTAATGAG	ACTTCTGAAA	4249
RATNaCh6A	TCCGGTTCGA	AATCGATATT	GTCAACAATA	AAACGGACTG	TGAGAAGCTC	4125
rPN4	TCCGGTTCGA	AATCGATATT	GTCAACAATA	AAACGGACTG	TGAGAAGCTC	4299
RATNaCh6A	ATGGAGGGCA	ACAGCACGGA	GATCCGATGG	AAGAATGTCA	AGATCAACTT	4175
rPN4	ATGGAGGGCA	ACAGCACGGA	GATCCGATGG	AAGAATGTCA	AGATCAACTT	4349
RATNaCh6A	TGACAATGTC	GGAGCAGGGT	ACCTGGCCCT	TCTTCAAGTG	GCAACCTTCA	4225
rPN4	TGACAATGTC	GGAGCAGGGT	ACCTGGCCCT	TCTTCAAGTG	GCAACCTTCA	4399

12/23

Fig. 3F: NaCh6/PN4 alignment (SEQ ID NO:7)

RATNaCh6A	AAGGCTGGAT	GGACATCATG	TATGCGGCTG	TAGATTCCCCG	AAAGCCAGAC	4275
rPN4	AAGGCTGGAT	GGACATCATG	TATGCGGCTG	TAGATTCCCCG	AAAGCCAGAC	4449
RATNaCh6A	GAGCAGCCTG	ACTACGAGGG	CAACATCTAC	ATGTACATCT	ACTTCGTCAT	4325
rPN4	GAGCAGCCTG	ACTACGAGGG	CAACATCTAC	ATGTACATCT	ACTTCGTCAT	4499
RATNaCh6A	CTTCATCATC	TTCGGCTCCT	TCTTCACCCT	CAACCTGTTC	ATCGGTGTCA	4375
rPN4	CTTCATCATC	TTCGGCTCCT	TCTTCACCCT	CAACCTGTTC	ATCGGTGTCA	4549
RATNaCh6A	TCATCGACAA	CTTCAACCAG	CAGAAGAAAA	AGTTTGGAGG	TCAGGACATC	4425
rPN4	TCATCGACAA	CTTCAACCAG	CAGAAGAAAA	AGTTTGGAGG	TCAGGACATC	4599
RATNaCh6A	TTCATGACAG	AGGAACAGAA	GAAGTACTAT	AATGCCATGA	AAAAGCTGGG	4475
rPN4	TTCATGACAG	AGGAACAGAA	GAAGTACTAC	AATGCCATGA	AAAAGCTGGG	4649
RATNaCh6A	CTCCAAGAAG	CCACAGAAGC	CCATCCCCCG	ACCCTTGAAC	AAAATCCAAG	4525
rPN4	CTCCAAGAAG	CCACAGAAGC	CCATCCCCCG	ACCCTTGAAC	AAAATCCAAG	4699
RATNaCh6A	GGATTGTCTT	TGATTTTCGTC	ACTCAACAAG	CCTTTGACAT	TGTGATCATG	4575
rPN4	GGATTGTCTT	TGATTTTCGTC	ACTCAACAAG	CCTTTGACAT	TGTGATCATG	4749
RATNaCh6A	ATGCTCATCT	GCCTTAACAT	GGTGACAATG	ATGGTGGAGA	CAGACACTCA	4625
rPN4	ATGCTCATCT	GCCTTAACAT	GGTGACAATG	ATGGTGGAGA	CAGACACTCA	4799
RATNaCh6A	GAGCAAGCAG	ATGGAGAACA	TTCTTTACTG	GATTAATCTG	GTCTTTGTCA	4675
rPN4	GAGCAAGCAG	ATGGAGAACA	TTCTTTACTG	GATTAATCTG	GTCTTTGTCA	4849
RATNaCh6A	TCTTCTTCAC	CTGCGAGTGT	GTGCTCAAAA	TGTTTGCCTT	GAGACACTAC	4725
rPN4	TCTTCTTCAC	CTGCGAGTGT	GTGCTCAAAA	TGTTTGCCTT	GAGACACTAC	4899
RATNaCh6A	TACTTCACCA	TTGGCTGGAA	CATCTTTGAC	TTTGTGGTGG	TCATCCTCTC	4775
rPN4	TATTTACCA	TTGGCTGGAA	CATCTTTGAC	TTTGTGGTGG	TCATCCTCTC	4949
RATNaCh6A	CATTGTGGGA	ATGTTCTCTG	CTGATATCAT	TGAGAAGTAC	TTCGTCTCCC	4825
rPN4	CATTGTGGGA	ATGTTCTCTG	CTGATATCAT	TGAGAAGTAC	TTCGTCTCCC	4999
RATNaCh6A	CAACCCTATT	CCGAGTTATC	CGATTGGCCC	GTATTGGGCG	CATCTTGCGT	4875
rPN4	CAACCCTATT	CCGAGTTATC	CGATTGGCCC	GTATTGGGCG	CATCTTGCGT	5049
RATNaCh6A	CTGATCAAGG	GCGCCAAAGG	GATCCGCACT	CTGCTCTTTG	CTCTGATGAT	4925
rPN4	CTGATCAAGG	GCGCCAAAGG	GATCCGCACC	CTGCTCTTTG	CCTTAATGAT	5099
RATNaCh6A	GTCGCTGCCC	GCCCTGTTCA	ACATCGGCCT	CCTGCTCTTC	CTCGTCATGT	4975
rPN4	GTCGCTGCCC	GCCCTGTTCA	ACATCGGCCT	CCTGCTCTTC	CTCGTCATGT	5149
RATNaCh6A	TCATCTTCTC	CATTTTTTGGC	ATGTCCAAC	TCGCATACGT	GAAGCACGAG	5025
rPN4	TCATCTTCTC	CATTTTTTGGC	ATGTCCAAC	TCGCATACGT	GAAGCACGAG	5199
RATNaCh6A	GCCGGCATTG	ACGACATGTT	CAACTTCGAG	ACATTTGGCA	ACAGCATGAT	5075
rPN4	GCCGGCATTG	ACGACATGTT	CAACTTCGAG	ACATTTGGCA	ACAGCATGAT	5249
RATNaCh6A	CTGTTTGTTC	CAGATCACAA	CGTCTGCTGG	CTGGGATGGC	CTGCTGCTGC	5125
rPN4	CTGTTTGTTC	CAGATCACAA	CGTCTGCTGG	CTGGGATGGC	CTGCTGCTGC	5299

13/23

Fig. 3G: NaCh6/PN4 alignment (SEQ ID NO:7)

RATNaCh6A	CAATCCTGAA	CCGCCCCCCT	GACTGCAGCT	TGGACAAAGA	GCACCCAGGG	5175
rPN4	CAATCCTGAA	CCGCCCCCCT	GACTGCAGCT	TGGACAAAGA	GCACCCAGGG	5349
RATNaCh6A	AGTGGCTTCA	AAGGGGACTG	TGGGAACCCC	TCGGTGGGCA	TCTTCTTCTT	5225
rPN4	AGTGGCTTCA	AAGGGGACTG	TGGGAACCCC	TCGGTGGGCA	TCTTCTTCTT	5399
RATNaCh6A	TGTGAGCTAC	ATCATCATCT	CCTTCCTGAT	TGTGGTGAAC	ATGTGCATCG	5275
rPN4	TGTGAGCTAC	ATCATCATCT	CCTTCCTGAT	TGTGGTGAAC	ATGTACATCG	5449
RATNaCh6A	CCATCATCCT	GGAGAACTTC	AGCGTGGCCA	CCGAGGAGAG	CGCCGACCCT	5325
rPN4	CCATCATCCT	GGAGAACTTC	AGCGTGGCCA	CCGAGGAGAG	CGCCGACCCT	5499
RATNaCh6A	CTGAGTGAGG	ATGACTTCGA	GACTTTCTAT	GAGATCTGGG	AGAAGTTTGA	5375
rPN4	CTGAGTGAGG	ATGACTTCGA	GACTTTCTAT	GAGATCTGGG	AGAAGTTTGA	5549
RATNaCh6A	CCCAGACGCC	ACCCAGTTCA	TCGAGTACTG	TAAGCTGGCA	GACTTTGCCG	5425
rPN4	CCCAGACGCC	ACCCAGTTCA	TCGAGTACTG	TAAGCTGGCA	GACTTTGCCG	5599
RATNaCh6A	ACGCCCTGGA	GCACCCGCTC	CGAGTACCCA	AGCCCAACAC	CATCGAGCTC	5475
rPN4	ACGCCCTGGA	GCACCCGCTC	CGAGTACCCA	AGCCCAACAC	CATCGAGCTC	5649
RATNaCh6A	ATCGCCATGG	ACCTGCCCCAT	GGTGAGCGGA	GATCGCATCC	ACTGCTTGGA	5525
rPN4	ATCGCCATGG	ACCTGCCCCAT	GGTGAGCGGA	GATCGCATCC	ACTGCTTGGA	5699
RATNaCh6A	CATCCTTTTC	GCCTTCACCA	AGGCAGTCCT	GGGAGACAGT	GGGGAGTTGG	5575
rPN4	CATCCTTTTC	GCCTTCACCA	AGGCAGTCCT	GGGAGACAGT	GGGGAGTTGG	5749
RATNaCh6A	ACATCCTGCG	GCAGCAGATG	GAGGAGCGGT	TCGTGGCATC	CAATCCTTCC	5625
rPN4	ACATCCTGCG	GCAGCAGATG	GAGGAGCGGT	TCGTGGCATC	CAATCCTTCC	5799
RATNaCh6A	AAAGTGTCTT	ACGAAGCCTA	TCAC-ACCAC	TCTGCGGCGC	AACGAGGAGG	5674
rPN4	AAAGTGTCTT	ACGA-GCCTA	TCACAACCAC	TCTGCGGCGC	AAGCAGGAGG	5848
RATNaCh6A	AGGTGTCTGC	AGTGGTCCTG	CAGCGTGCCT	ACAGGGGACA	CTTGGCTAGG	5724
rPN4	AGGTGTCTGC	AGTGGTCCTG	CAGCGTGCCT	ACAGGGGACA	CTTGGCTAGG	5898
RATNaCh6A	CGGGGCTTCA	TCTGCAGAAA	GATGGCCTCC	AACAAGCTGG	AGAATGGAGG	5774
rPN4	CGGGGCTTCA	TCTGCAGAAA	GATGGCCTCC	AACAAGCTGG	AGAATGGAGG	5948
RATNaCh6A	CACACACAGA	GACAAGAAGG	AGAGCACCCC	GTCCACAGCC	TCCCTCCCCCT	5824
rPN4	CACACACAGA	GACAAGAAGG	AGAGCACCCC	GTCCACAGCC	TCCCTCCCCCT	5998
RATNaCh6A	CTTACGACAG	CCTCACAAAG	CCAGACAAGG	AGAAGCAGCA	GCGTGCGGAG	5874
rPN4	CTTACGACAG	CCTCACAAAG	CCAGACAAGG	AGAAGCAGCA	GCGTGCGGAG	6048
RATNaCh6A	GAGGGCAGAA	GGGAAAGAGC	CAAGAGGCAA	AAAGAGGTCA	GGGAGTCCAA	5924
rPN4	GAGGGCAGAA	GGGAAAGAGC	CAAGAGGCAA	AAAGAGGTCA	GGGAGTCCAA	6098
	stop					
RATNaCh6A	GTGCTAGAGG	AGGGGAAAGG	AAGCTTACCC	CGGCTGAACA	CTGGCAAGTG	5974
rPN4	GTGCTAGAGG	AGGGGAAAGG	AAGCTTACCC	CGGCTGAACA	CTGGCAAGTG	6148
RATNaCh6A	AAAGCTTGTT	TACAAACTTC	CGAATCTCAC	GGATGCAGAG	CAGCTGTGCA	6023
rPN4	AAAGCTTGTT	TACAAACTTC	CGAATCTCAC	GGATGCAGAG	CAGCTGTGCA	6198

14/23

Fig. 3H: NaCh6/PN4 alignment (SEQ ID NO:7)

RATNaCh6A	GACGCTCGCT	GTACTGGAAG	ACCTATACCA	AACATAGTCT	GCTTACATGT	6073
rPN4	GACGCTCGCT	GTACTGGAAG	ACCTATACCA	AACATAGTCT	GCTTACATGT	6248
RATNaCh6A	GACATGGTGG	CATCCTGAGC	GGTGACTGCT	GCTGGGGACA	AAGGACCCTG	6123
rPN4	GACATGGTGG	CATCCTGAGC	GGTGA---CT	GCTGGGGACA	AAGGACCCTG	6295
RATNaCh6A	CTCCCTGGAC	TCACAGATCT	CCTATCGCTT	GGGCAGACGG	TTACTGCATG	6173
rPN4	CTCCCTGGAC	TCACAGATCT	CCTATCGCTT	GGGCAGACGG	TTACTGCATG	6345
RATNaCh6A	TTCCACACTT	AGTCAATGCA	ACTTAGGACT	AAACTAACCA	GGATACAAAA	6223
rPN4	TTCCACACTT	AGTCAATGCA	ACTTAGGACT	AAACTAACCA	GGATACAAAA	6395
RATNaCh6A	CCGAGGCGGC	TG--GCGACC	AGCAGATCAC	CGCTGCAGCC	AAATGGATTT	6271
rPN4	CCGAGGCGGC	TGCCGGGACC	AGCAGATCAC	CGCTGCAGCC	AAATGGATTT	6445
RATNaCh6A	TATTTTTTCA	TTTTGTTGAT	TCTCAGAAGC	AGAAAGCATC	ACTTTAAAAG	6321
rPN4	TATTTTTTCA	TTTTGTTGAT	TCTCAGAAGC	AGAAAGCATC	ACTTTAAAAG	6495
RATNaCh6A	TTTGTGTTGT	CATGCAAACA	ATATTTGAAT	TCTTACATTA	GTAAAGCTAA	6371
rPN4	TTTGTGTTGT	CATNCAAACA	ATATTTGAAT	TCTTACATTA	GTAAAGCTAA	6545
RATNaCh6A	GCAGCAAAAA	GAAACACACA	CGCACACAGA	CACACAAAGA	CACACACACA	6421
rPN4	GCANCAAAAA	G-----	-----	-----	-----	6556
RATNaCh6A	TTCAGCCTAT	GTCACCTAATC	GTCTGTTTCT	TTAACATAAC	AGCATCTTCT	6471
rPN4	-----	-----	-----	-----	-----	6556
RATNaCh6A	CCACACGAGC	GGCACGTGGT	TTGGAGATGG	GTGGGGGAAA	ATCAGGGTTT	6521
rPN4	-----	-----	-----	-----	-----	6556
RATNaCh6A	CAGGCTGAGG	AGGACTTGCT	CAGGCCAATC	CCAAATATGT	GCTCGTTCAA	6571
rPN4	-----	-----	-----	-----	-----	6556
RATNaCh6A	TGCATAGAAG	TGACCTGCAT	GATGGCATGC	TGTGTTTCAGA	AGTCATGCAT	6621
rPN4	-----	-----	-----	-----	-----	6556
RATNaCh6A	GAGACCCACA	CACCACAAGA	CACTAGTACT	CCTGTNNCCA	TCCACAGGCT	6671
rPN4	-----	-----	-----	-----	-----	6556
RATNaCh6A	CAGCCTGCGG	ACAGGACCAG	CCCTGCACCG	TTCCTGTAT	TTGGAGAAAT	6721
rPN4	-----	-----	-----	-----	-----	6556
RATNaCh6A	GGTAAGAGTT	CCACACCGGC	TGCAGTCCTC	TCAGTGTAGG	ATTCTTTTCGT	6771
rPN4	-----	-----	-----	-----	-----	6556
RATNaCh6A	ACACCTCTGG	GTAGGGAGAC	ATAATTAACC	AATTGACCAC	TACCAACAAA	6821
rPN4	-----	-----	-----	-----	-----	6556
RATNaCh6A	ACAAT	6825				
rPN4	-----	6556				

15/23

Fig. 4A: PN4a/PN4/NaCh6 alignment

rPN4a	M--AARLLAP	PGPDSFKPFT	PESLANIERR	IAESKLKKPP	KADGSHREDD	48
rPN4	M--AARLLAP	PGPDSFKPFT	PESLANIERR	IAESKLKKPP	KADGSHREDD	48
RATNaCh6A	MRRSARLLAP	PGPDSFKPFT	PESLANIERR	IAESKLKKPP	KADGSHREDD	50
rPN4a	EDSKPKPNSD	LEAGKSLPFI	YGDIPQGLVA	VPLEDFDPYY	LTQKTFVVLN	98
rPN4	EDSKPKPNSD	LEAGKSLPFI	YGDIPQGLVA	VPLEDFDPYY	LTQKTFVVLN	98
RATNaCh6A	EDSKPKPNSD	LEAGKSLPFI	YGDIPQGLVA	VPLEDFDPYY	LTQKTFVVLN	100
[-----IS1-----]						
rPN4a	RGKTLFRFSA	TPALYILSPF	NLIRRIAII	LIHSVFSMII	MCTILTNCVF	148
rPN4	RGKTLFRFSA	TPALYILSPF	NLIRRIAII	LIHSVFSMII	MCTILTNCVF	148
RATNaCh6A	RGKTLFRFSA	TPALYILSPF	NLIRRIAII	LIHSVFSMII	MCTILTNCVF	150
-] [-----IS2-----] [-----]						
rPN4a	MTFSNPPEWS	KNVEYTFTGI	YTFESLVKII	ARGFCIDGFT	FLRDPWNWLD	198
rPN4	MTFSNPPEWS	KNVEYTFTGI	YTFESLVKII	ARGFCIDGFT	FLRDPWNWLD	198
RATNaCh6A	MTFSNPPEWS	KNVEYTFTGI	YTFESLVKII	ARGFCIDGFT	FLRDPWNWLD	200
--IS3---] [-----IS4-----]						
rPN4a	FSVIMMAYVT	EFVDLGNVSA	LRTFRVLRAL	KTISVIPGLK	TIVGALIQSV	248
rPN4	FSVIMMAYVT	EFVDLGNVSA	LRTFRVLRAL	KTISVIPGLK	TIVGALIQSV	248
RATNaCh6A	FSVIMMAYVT	EFVDLGNVSA	LRTFRVLRAL	KTISVIPGLK	TIVGALIQSV	250
[-----IS5-----]						
rPN4a	KKLSDDVMILT	VFCLSVFALI	GLQLFMGNLR	NKCVVWPINF	NESYLENGTR	298
rPN4	KKLSDDVMILT	VFCLSVFALI	GLQLFMGNLR	NKCVVWPINF	NESYLENGTR	298
RATNaCh6A	KKLSDDVMILT	VFCLSVFALI	GLQLFMGNLR	NKCVVWPINF	NESYLENGTR	300
rPN4a	GFDWEEYINN	KTNFYMVPGM	LEPLLCGNSS	DAGQCPEGFQ	CMKAGRNPNY	348
rPN4	GFDWEEYINN	KTNFYMVPGM	LEPLLCGNSS	DAGQCPEGFQ	CMKAGRNPNY	348
RATNaCh6A	GFDWEEYINN	KTNFYMVPGM	LEPLLCGNSS	DAGQC-EGFQ	CSKAGRNPNY	349
[-----IS6-----]						
rPN4a	GYTSFDTFSW	AFLALFRLMT	QDYWENLYQL	TLRAAGKTYM	IFFVLVIFVG	398
rPN4	GYTSFDTFSW	AFLALFRLMT	QDYWENLYQL	TLRAAGKTYM	IFFVLVIFVG	398
RATNaCh6A	GYTSFDTFSW	AFLALFRLMT	QDYWENLYQL	TLRAAGKTYM	IFFVLVIFVG	399
-----]						
rPN4a	SFYLVNLILA	VVAMAYEEQN	QATLEEAQK	EAEFKAMLEQ	LKKQQEEAQA	448
rPN4	SFYLVNLILA	VVAMAYEEQN	QATLEEAQK	EAEFKAMLEQ	LKKQQEEAQA	448
RATNaCh6A	SFYLVNLILA	VVAMAYEEQN	QATLEEAQK	EAEFKAMLEQ	LKKQQEEAQA	449
rPN4a	AAMATSAGTV	SEDAIEEEGE	DGVGSPRSSS	ELSKLSSKSA	KERRNRRKKR	498
rPN4	AAMATSAGTV	SEDAIEEEGE	DGVGSPRSSS	ELSKLSSKSA	KERRNRRKKR	498
RATNaCh6A	AAMATSAGTV	SEDAIEEEGE	DGVGSPRSSS	ELSKLSSKSA	KERRNRRKKR	499
rPN4a	KQKELSEGEE	KGDPEKVFKS	ESEDGMRRKA	FRLPDNRIGR	KFSIMNQSL	548
rPN4	KQKELSEGEE	KGDPEKVFKS	ESEDGMRRKA	FRLPDNRIGR	KFSIMNQSL	548
RATNaCh6A	KQKELSEGEE	KGDPEKVFKS	ESEYGMRRKA	FRLPDNRIGR	KFSIMNQSL	549
rPN4a	SIPGSPFLSR	HNSKSSIFSF	RGPGRFRDPG	SENEFADDEH	STVEESEGR	598
rPN4	SIPGSPFLSR	HNSKSSIFSF	RGPGRFRDPG	SENEFADDEH	STVEESEGR	598
RATNaCh6A	SIPGSPFLSR	HNSKSSIFSF	GDPS-VRDPG	SENEFADDEH	STVEESEGR	598

16/23

Fig. 4B: PN4a/PN4/NaCh6 alignment

rPN4a	DSLFIPIRAR	ERRSSYSGYS	GYSQCSRSSR	IFPSLRRSVK	RNSTVDCNGV	648
rPN4	DSLFIPIRAR	ERRSSYSGYS	GYSQCSRSSR	IFPSLRRSVK	RNSTVDCNGV	648
RATNaCh6A	DSLFIPIRAR	ERRSSYSGYS	GYSQCSRSSR	ISPACAQR-E	ANSTVDCNGV	647
rPN4a	VSLIGPGSHI	GRLLEPKID	KAATDSATTE	VEIKKKGPGS	LLVSMDQLAS	698
rPN4	VSLIGPGSHI	GRLLE----	-----ATTE	VEIKKKGPGS	LLVSMDQLAS	688
RATNaCh6A	VSLIGPGSHI	GRLLLR----	-----QRLR	WKLRRKALDS	-FSFYGPTRL	686
rPN4a	YGRKDRINSI	MSVVTNTLVE	ELEESQRKCP	PCWYKFANTF	LIWECHPYWI	748
rPN4	YGRKDRINSI	MSVVTNTLVE	ELEESQRKCP	PCWYKFANTF	LIWECHPYWI	738
RATNaCh6A	LRTEGQNQQH	NERGHKHA	ELEESQRKCP	PCWYKFANTF	LIWECHPYWI	736
	[-----IIS1-----]					
rPN4a	KLKEIVNLIV	MDPFVDLAIT	ICIVLNTLFM	AMEHHPMTPQ	FEHVLAVGNL	798
rPN4	KLKEIVNLIV	MDPFVDLAIT	ICIVLNTLFM	AMEHHPMTPQ	FEHVLAVGNL	788
RATNaCh6A	KLKEIVNLIV	MDPFVDLAIT	ICIVLNTLFM	AMEHHPMTPQ	FEHVLAVGNL	786
	--IIS2-----]					
	[-----IIS3-----]					
rPN4a	VFTGIFTAEM	FLKLIAMDPY	YYFQEGWNIF	DGFIVSLSLM	ELSLADVEGL	848
rPN4	VFTGIFTAEM	FLKLIAMDPY	YYFQEGWNIF	DGFIVSLSLM	ELSLADVEGL	838
RATNaCh6A	VFTGIFTAEM	FLKLIAMDPY	YYFQEGWNIF	DGFIVSLSLM	ELSLADVEGL	836
	[-----IIS4-----]					
	[-----IIS5-----]					
rPN4a	SVLRSFRLLR	VFKLAKSWPT	LNMLIKIIGN	SVGALGNLTL	VLAIIVFIFA	898
rPN4	SVLRSFRLLR	VFKLAKSWPT	LNMLIKIIGN	SVGALGNLTL	VLAIIVFIFA	888
RATNaCh6A	SVLRSFRLLR	VFKLAKSWPT	LNMLIKIIGN	SVGALGNLTL	VLAIIVFIFA	886
	-----]					
rPN4a	VVGMQLFGKS	YKECVCKINQ	ECKLPRWHMN	DDFHSFLIVF	RVLCGEWIET	948
rPN4	VVGMQLFGKS	YKECVCKINQ	ECKLPRWHMN	DDFHSFLIVF	RVLCGEWIET	938
RATNaCh6A	VVGMQLFGKS	YKECVCKINQ	ECKLPRWHMN	DDFHSFLIVF	RVLCGEWIET	936
	[-----IIS6-----]					
rPN4a	MWDCMEVAGQ	AMCLIVFMMV	MVIGNLVVLN	LFLALLLSSF	SADNLAATDD	998
rPN4	MWDCMEVAGQ	AMCLIVFMMV	MVIGNLVVLN	LFLALLLSSF	SADNLAATDD	988
RATNaCh6A	MWDCMEVAGQ	AMCLIVFMMV	MVIGNLVVLN	LFLALLLSSF	SADNLAATDD	986
rPN4a	DGEMNNLQIS	VIRIKKGVAV	TKVKVHAFMQ	AHFKQREADE	VKPLDELYEK	1048
rPN4	DGEMNNLQIS	VIRIKKGVAV	TKVKVHAFMQ	AHFKQREADE	VKPLDELYEK	1038
RATNaCh6A	DGEMNNLQIS	VIRIKKGVAV	TKVKVHAFMQ	AHFKQREADE	VKPLDELYEK	1036
rPN4a	KANCIAHNTG	VDIHRNGDFQ	KNGNGTTSI	GSSVEKYIID	EDHMSFINNP	1098
rPN4	KANCIAHNTG	VDIHRNGDFQ	KNGNGTTSI	GSSVEKYIID	EDHMSFINNP	1088
RATNaCh6A	KANCIAHNTG	VDIHRNGDFQ	KNGNGTTSI	GSSVEKYIID	EDHMSFINNP	1086
rPN4a	NLTVRVPIAV	GESDFENLNT	EDVSSSEDPE	GSKDKLDDTS	SSEGSTIDIK	1148
rPN4	NLTVRVPIAV	GESDFENLNT	EDVSSSEDPE	GSKDKLDDTS	SSEGSTIDIK	1138
RATNaCh6A	NLTVRVPIAV	GESDFENLNT	EDVSSSEDPE	GSKDKLDDTS	SSEGSTIDIK	1136
rPN4a	PEVEEVPEEQ	PEEYLDPDAC	FTEGCVQRFK	CCQVNIIEGL	GKSWWILRKT	1198
rPN4	PEVEEVPEEQ	PEEYLDPDAC	FTEGCVQRFK	CCQVNIIEGL	GKSWWILRKT	1188
RATNaCh6A	PEVEEVPEEQ	PEEYLDPDAC	FTEGCVQRFK	CCQVNIIEGL	GKSWWILRKT	1186

17/23

Fig. 4C: PN4a/PN4/NaCh6 alignment

	[-----IIIS1-----]					[-----IIIS2					
rPN4a	CFLIVEHWNF	ETFIIFMILL	SSGALAFEDI	YIEQRKTIRT	ILEYADKVFT						1248
rPN4	CFLIVEHWNF	ETFIIFMILL	SSGALAFEDI	YIEQRKTIRT	ILEYADKVFT						1238
RATNaCh6A	CFLIVEHWNF	ETFIIFMILL	SSGALAFEDI	YIEQRKTIRT	ILEYADKVFT						1236
	-----]					[-----IIIS3-----]					
rPN4a	YIFILEMLLK	WTAYGFVKFF	TNAWCWLDL	IVAVSLVSLI	ANALGYSELG						1298
rPN4	YIFILEMLLK	WTAYGFVKFF	TNAWCWLDL	IVAVSLVSLI	ANALGYSELG						1288
RATNaCh6A	YIFILEMLLK	WTYGFVKFF	TNAWCWLDL	IVAVSLVSLI	ANALGYSELG						1286
	-----IIIS4-----]					[-----IIIS5					
rPN4a	AIKSLRTLRA	LRPLRALSRF	EGMRVVVNAL	VGAIPSIMNV	LLVCLIFWLI						1348
rPN4	AIKSLRTLRA	LRPLRALSRF	EGMRVVVNAL	VGAIPSIMNV	LLVCLIFWLI						1338
RATNaCh6A	AIKSLRTLRA	LRPLRALSRF	EGMRVVVNAL	VGAIPSIMNV	LLVCLIFWLI						1336
	-----]										
rPN4a	FSIMGVNLFA	GKYHYCFNET	SEIRFEIDIV	NNKTDCEKLM	EGNSTEIRWK						1398
rPN4	FSIMGVNLFA	GKYHYCFNET	SEIRFEIDIV	NNKTDCEKLM	EGNSTEIRWK						1388
RATNaCh6A	FSIMGVNLFA	GKYHYCFNET	SEIRFEIDIV	NNKTDCEKLM	EGNSTEIRWK						1386
						[---					
rPN4a	NVKINFNVG	AGYLALLQVA	TFKGWMDIMY	AAVDSRKPDE	QPDYEGNIYM						1448
rPN4	NVKINFNVG	AGYLALLQVA	TFKGWMDIMY	AAVDSRKPDE	QPDYEGNIYM						1438
RATNaCh6A	NVKINFNVG	AGYLALLQVA	TFKGWMDIMY	AAVDSRKPDE	QPDYEGNIYM						1436
	-----IIIS6-----]										
rPN4a	YIYFVIFIIF	GSFFTLLNLF	GVIIDNFNQ	KKKFGGQDIF	MTEEQKKYYN						1498
rPN4	YIYFVIFIIF	GSFFTLLNLF	GVIIDNFNQ	KKKFGGQDIF	MTEEQKKYYN						1488
RATNaCh6A	YIYFVIFIIF	GSFFTLLNLF	GVIIDNFNQ	KKKFGGQDIF	MTEEQKKYYN						1486
						[-----IVS1-----]					
rPN4a	AMKKLGSKKP	QKPIPRPLNK	IQGIVDFVT	QQAQDIVIMM	LICLNMTMM						1548
rPN4	AMKKLGSKKP	QKPIPRPLNK	IQGIVDFVT	QQAQDIVIMM	LICLNMTMM						1538
RATNaCh6A	AMKKLGSKKP	QKPIPRPLNK	IQGIVDFVT	QQAQDIVIMM	LICLNMTMM						1536
	[-----IVS2-----]					[-----					
rPN4a	VETDTQSKQM	ENILYWINLV	FVIFFTCECV	LKMFALRHYY	FTIGWNIFDF						1598
rPN4	VETDTQSKQM	ENILYWINLV	FVIFFTCECV	LKMFALRHYY	FTIGWNIFDF						1588
RATNaCh6A	VETDTQSKQM	ENILYWINLV	FVIFFTCECV	LKMFALRHYY	FTIGWNIFDF						1586
	--IVS3-----]					[-----IVS4-----]					
rPN4a	VVILSIVGM	FLADIIEKYF	VSPTLFRVIR	LARIGRILRL	IKGAKGIRTL						1648
rPN4	VVILSIVGM	FLADIIEKYF	VSPTLFRVIR	LARIGRILRL	IKGAKGIRTL						1638
RATNaCh6A	VVILSIVGM	FLADIIEKYF	VSPTLFRVIR	LARIGRILRL	IKGAKGIRTL						1636
	[-----IVS5-----]										
rPN4a	LFALMMSLPA	LFNIGLLLF	VMFIFSIFGM	SNFAYVKHEA	GIDDMFNFT						1693
rPN4	LFALMMSLPA	LFNIGLLLF	VMFIFSIFGM	SNFAYVKHEA	GIDDMFNFT						1688
RATNaCh6A	LFALMMSLPA	LFNIGLLLF	VMFIFSIFGM	SNFAYVKHEA	GIDDMFNFT						1686
rPN4a	FGNSMICLFQ	ITTSAGWDGL	LLPILNRPPD	CSLDKEHPGS	GFKGDCGNPS						1748
rPN4	FGNSMICLFQ	ITTSAGWDGL	LLPILNRPPD	CSLDKEHPGS	GFKGDCGNPS						1738
RATNaCh6A	FGNSMICLFQ	ITTSAGWDGL	LLPILNRPPD	CSLDKEHPGS	GFKGDCGNPS						1736

18/23

Fig. 4D: PN4a/PN4/NaCh6 alignment

	[-----IVS6-----!]						
rPN4a	VGIFFFVSYI	IISFLIVVM	YIAIILENFS	VATEESADPL	SEDDFETFYE		1798
rPN4	VGIFFFVSYI	IISFLIVVM	YIAIILENFS	VATEESADPL	SEDDFETFYE		1788
RATNaCh6A	VGIFFFVSYI	IISFLIVVM	CIAIILENFS	VATEESADPL	SEDDFETFYE		1786
rPN4a	IWEKFDPDAT	QFIEYCKLAD	FADALEHPLR	VPKPNTIELI	AMDLPMSVSGD		1848
rPN4	IWEKFDPDAT	QFIEYCKLAD	FADALEHPLR	VPKPNTIELI	AMDLPMSVSGD		1838
RATNaCh6A	IWEKFDPDAT	QFIEYCKLAD	FADALEHPLR	VPKPNTIELI	AMDLPMSVSGD		1836
rPN4a	RIHCLDILFA	FTKRVLGDSG	ELDILRQQME	ERFVASNPSK	VSYPEITTTTL		1898
rPN4	RIHCLDILFA	FTKRVLGDSG	ELDILRQQME	ERFVASNPSK	VSYPEITTTTL		1888
RATNaCh6A	RIHCLDILFA	FTKAVLGDSG	ELDILRQQME	ERFVASNPSK	VSYEAYHTTL		1886
rPN4a	RRKQEEVSAV	VLQRAYRGHL	ARRGFICRKM	ASNKLENGGT	HRDKKESTPS		1948
rPN4	RRKQEEVSAV	VLQRAYRGHL	ARRGFICRKM	ASNKLENGGT	HRDKKESTPS		1938
RATNaCh6A	RRNEEEVSAV	VLQRAYRGHL	ARRGFICRKM	ASNKLENGGT	HRDKKESTPS		1936
rPN4a	TASLPSYDSV	TKPDKEKQQR	AEEGRRERAK	RQKEVRESKC		1988	
rPN4	TASLPSYDSV	TKPDKEKQQR	AEEGRRERAK	RQKEVRESKC		1978	
RATNaCh6A	TASLPSYDSV	TKPDKEKQQR	AEEGRRERAK	RQKEVRESKC		1976	

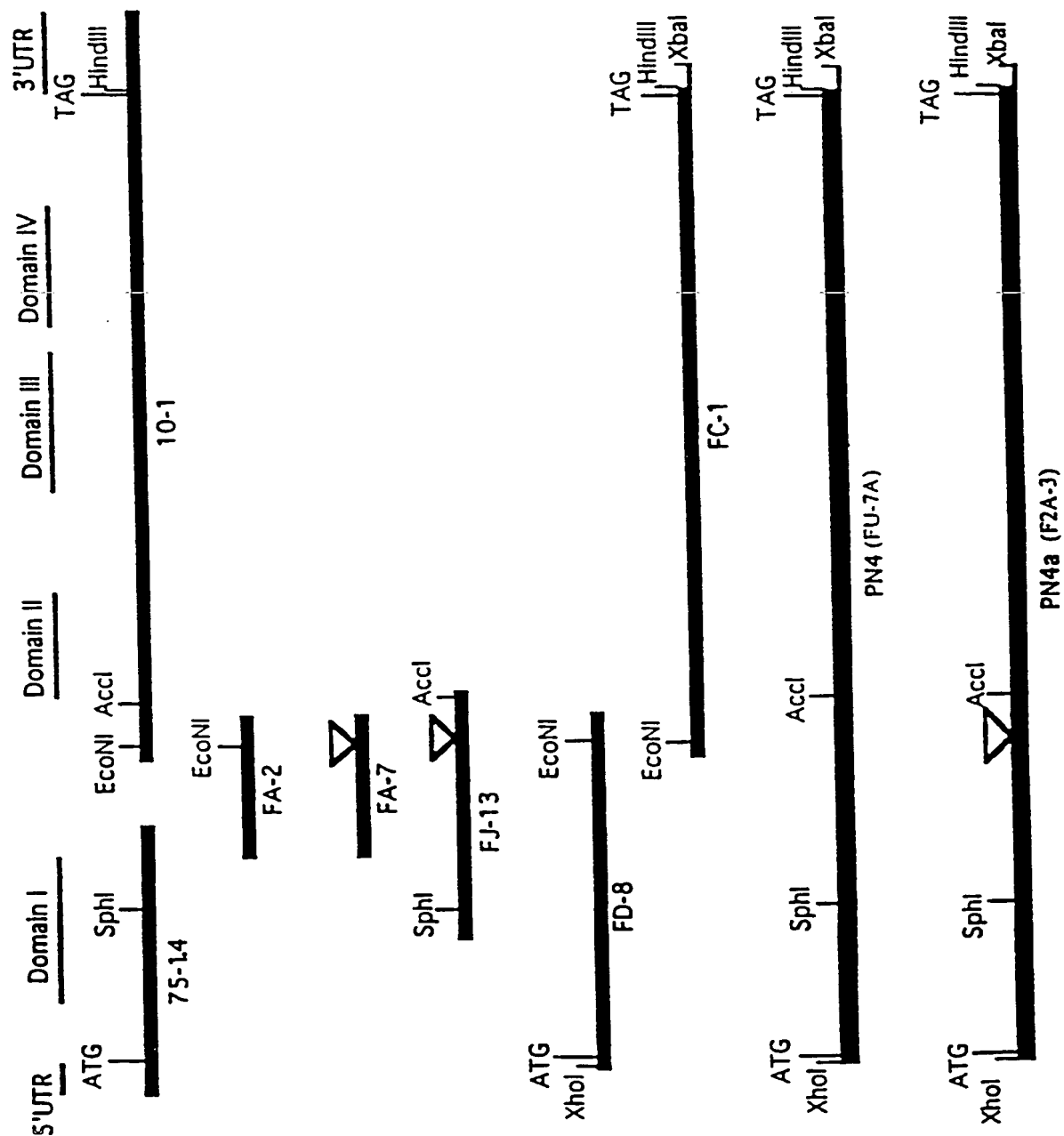
19/23

Fig. 5: PN4a/PN4/NaCh6/BrainII Interdomain I/II region comparison

rPN4a	DSLFIPIRAR	ERRSSYSGYS	GYSQCSRSSR	IFPSLRRSVK	RNSTVDCNGV	648
rPN4	DSLFIPIRAR	ERRSSYSGYS	GYSQCSRSSR	IFPSLRRSVK	RNSTVDCNGV	648
RATNaCh6A	DSLFIPIRAR	ERRSSYSGYS	GYSQCSRSSR	ISPACAQR-E	ANSTVDCNGV	647
rBrainII	DSLFPVPEREG	ERRP-----S	NVSQASRASR	GIPTLPMNGK	MHSADVDCNGV	653
rPN4a	VSLIGPGSHI	----GRLLPEVKID	KAATDSATT-E	VEIKKKGPGS	LLVSMDQLAS	698
rPN4	VSLIGPGSHI	----GRLLPE----	-----ATT-E	VEIKKKGPGS	LLVSMDQLAS	688
RATNaCh6A	VSLIGPGSHI	----GRLLLR----	-----QRL-R	WKLRRKALDS	-FSFYGPTRL	686
rBrainII	VSLVGGPSAL	TSPVGQLLPE----	-----GTTTE	TEIRKRRSSS	YHVSMDLLED	698
rPN4a	YGRKDRINSI	MSVVTNTLVE	ELEESQRKCP	PCWYKFANTF	LIWECHPYWI	748
rPN4	YGRKDRINSI	MSVVTNTLVE	ELEESQRKCP	PCWYKFANTF	LIWECHPYWI	738
RATNaCh6A	LRTEGQNQOH	NERGHKHAEE	ELEESQRKCP	PCWYKFANTF	LIWECHPYWI	736
rBrainII	PSR-QRAMSI	ASILTNTM-E	ELEESRQKCP	PCWYKFANMC	LIWDCCKPWL	746

20/23

Fig. 6



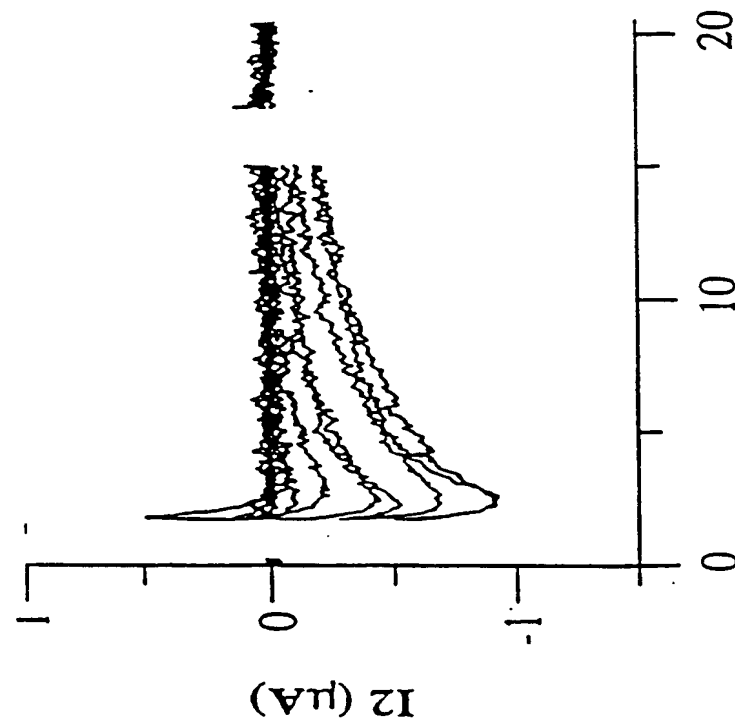


Fig. 7(a)

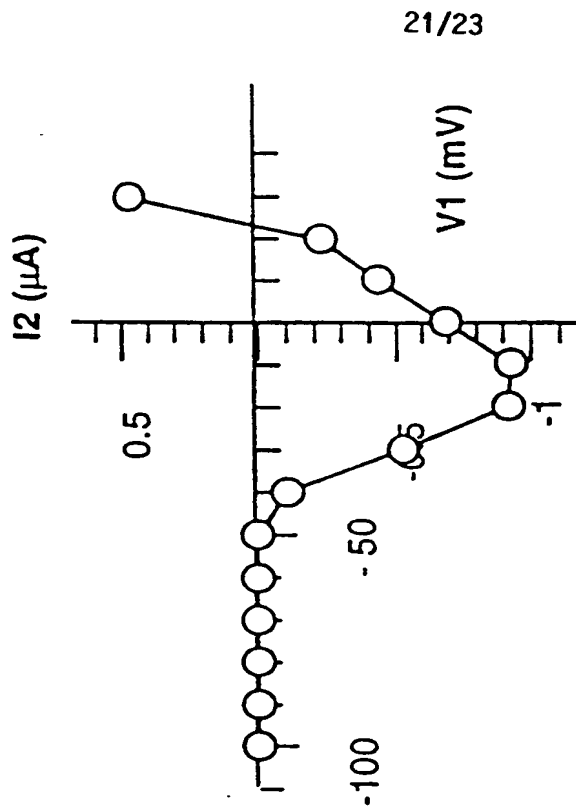


Fig. 7(b)

22/23

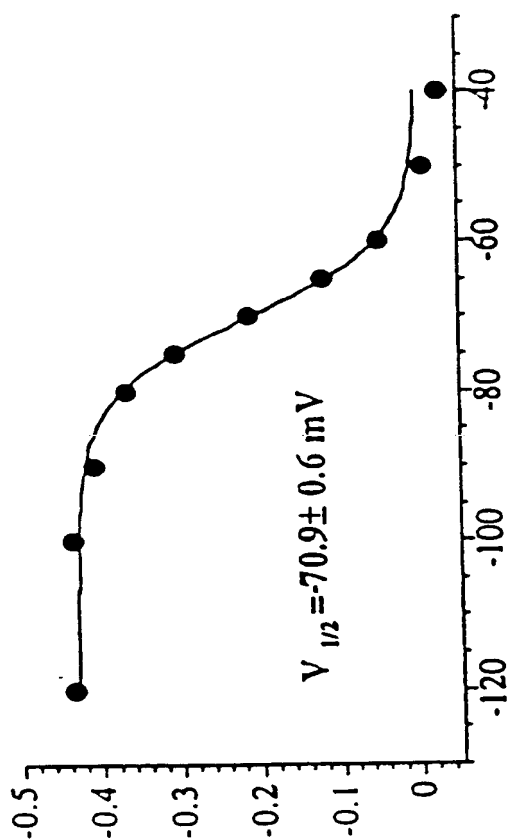


Fig. 8(b)

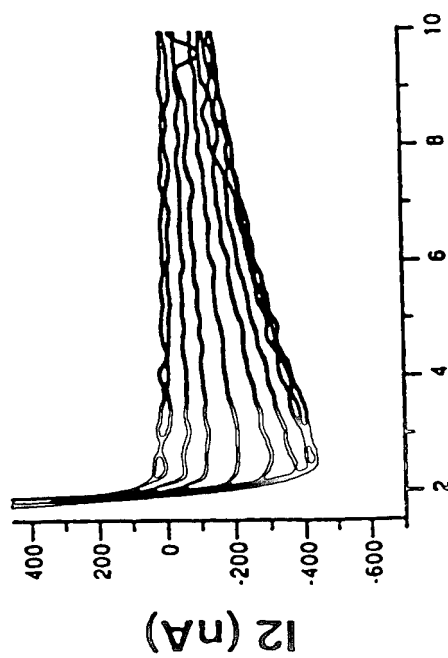


Fig. 8(a)

23/23

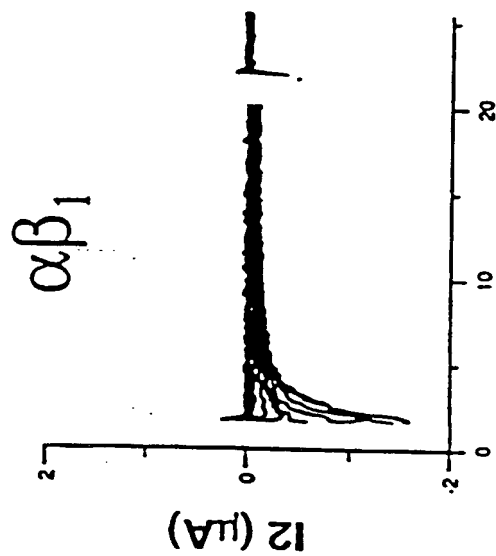


Fig. 9(a)

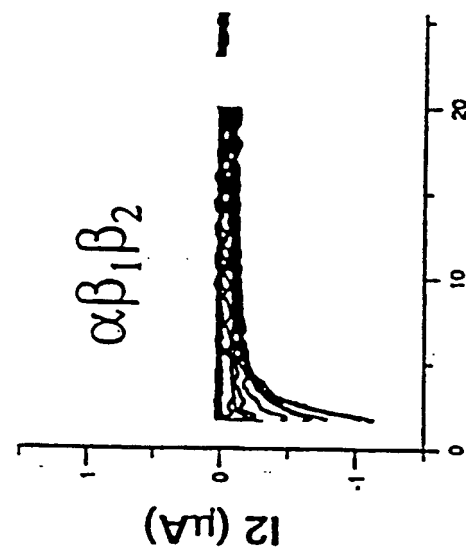


Fig. 9(b)

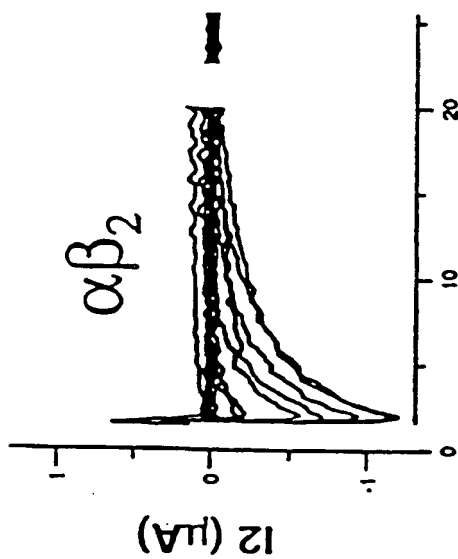


Fig. 9(c)

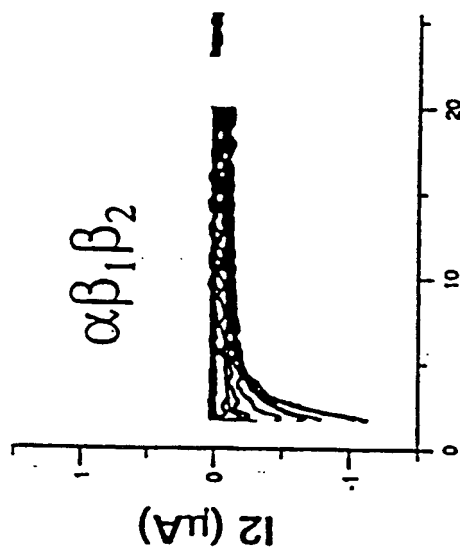


Fig. 9(d)



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/12, C07K 14/705, 16/28, C12N 5/10, 1/21, C12Q 1/68	A3	(11) International Publication Number: WO 98/38302 (43) International Publication Date: 3 September 1998 (03.09.98)
(21) International Application Number: PCT/EP98/00997 (22) International Filing Date: 20 February 1998 (20.02.98) (30) Priority Data: 60/039,447 26 February 1997 (26.02.97) US (71) Applicant: F. HOFFMANN-LA ROCHE AG [CH/CH]; Grenzacherstrasse 124, CH-4070 Basle (CH). (72) Inventors: DELGADO, Stephen, Gregory; Apartment #3, 358 25th Avenue, San Francisco, CA 94121 (US). DIETRICH, Paul, Shartzer; 3949 Bibbits Drive, Palo Alto, CA 94303 (US). FISH, Linda, Marie; Star Route 2, Box 327-A, La Honda, CA 94020 (US). HERMAN, Ronald, Charles; 467-D Costa Mesa Terrace, Sunnyvale, CA 94086 (US). SANGAMESWARAN, Lakshmi; 350 Avenida Arboles, San Jose, CA 95123 (US). (74) Agent: MEZGER, Wolfgang; Grenzacherstrasse 124, CH-4070 Basle (CH).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> (88) Date of publication of the international search report: 3 December 1998 (03.12.98)
(54) Title: TETRODOTOXIN-SENSITIVE SODIUM CHANNEL α -SUBUNIT (57) Abstract <p>DNA encoding for a voltage-gated, TTX-sensitive sodium channel is isolated. Also disclosed are polypeptide products of recombinant expression of these DNA sequences, expression vectors comprising the DNA sequence, and host cells transformed with these expression vectors. Other aspects of this invention are peptides whose sequences are based on the amino acid sequences deduced from these DNA sequences, antibodies specific for such proteins and peptides, procedures for detection and quantitation of such proteins and nucleic acids related thereto. Another aspect of this invention is the use of this voltage-gated, tetrodotoxin-sensitive sodium channel as a therapeutic target for compounds.</p>		

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INTERNATIONAL SEARCH REPORT

Int. l. Application No

PCT/EP 98/00997

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/705 C07K16/28 C12N5/10 C12N1/21
C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KLUGBAUER N ET AL: "Structure and functional expression of a new member of the tetrodotoxin-sensitive voltage-activated sodium channel family from human neuroendocrine cells." EMBO J, MAR 15 1995, 14 (6) P1084-90, XP002069908 ENGLAND see abstract; figure 1 ---	1-50
Y	SCHALLER KL ET AL: "A novel, abundant sodium channel expressed in neurons and glia." J NEUROSCI, MAY 1995, 15 (5 PT 1) P3231-42, XP002069909 UNITED STATES see abstract; figure 2 ---	1-37, 45-50



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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Date of the actual completion of the international search

3 September 1998

Date of mailing of the international search report

11.09.1998

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Fax: (+31-70) 340-3016

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Gurdjian, D

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 98/00997

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 380 836 A (ROGART RICHARD B) 10 January 1995 see examples 7,10,11,14 ----	1-37, 45-50
Y	US 5 439 808 A (BLAKE MILAN S ET AL) 8 August 1995 see column 15, paragraph 2 ----	38-44
Y	EP 0 483 113 A (BIO TECHNOLOGY GENERAL CORP) 29 April 1992 see examples 5,13,24 -----	38-44

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 98/00997

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2. ☐ Claims Nos.:
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INTERNATIONAL SEARCH REPORT

International Application No. PCT/EP 98/00997

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This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 50 partly 1-37, 45-49

tetrodotoxin-sensitive sodium channel DNA sequences proteins with seq.id.1-7 ,vectors, hosts, assays and antibodies .

2. Claims: 50 partly,38-44

method of growing plasmids containing constructs of tetrodotoxin-sensitive sodium channel proteins employing competent E.coli .

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. l. Application No

PCT/EP 98/00997

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